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**Nagy et al.**

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- (54) **ENZYMATIC HYDROLYSIS OF DISACCHARIDES AND OLIGOSACCHARIDES USING ALPHA-GLUCOSIDASE ENZYMES**
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(57) **ABSTRACT**

A method is disclosed for hydrolyzing an alpha-1,3 or alpha-1,6 glucosyl-glucose linkage in a saccharide (disaccharide or oligosaccharide). This method comprises contacting the saccharide with an alpha-glucosidase enzyme such as transglucosidase under suitable conditions, during which contacting step the enzyme hydrolyzes at least one alpha-1,3 or alpha-1,6 glucosyl-glucose linkage of the saccharide. This method is useful for reducing the amount of oligosaccharides in a filtrate isolated from a glucan synthesis reaction, for example.

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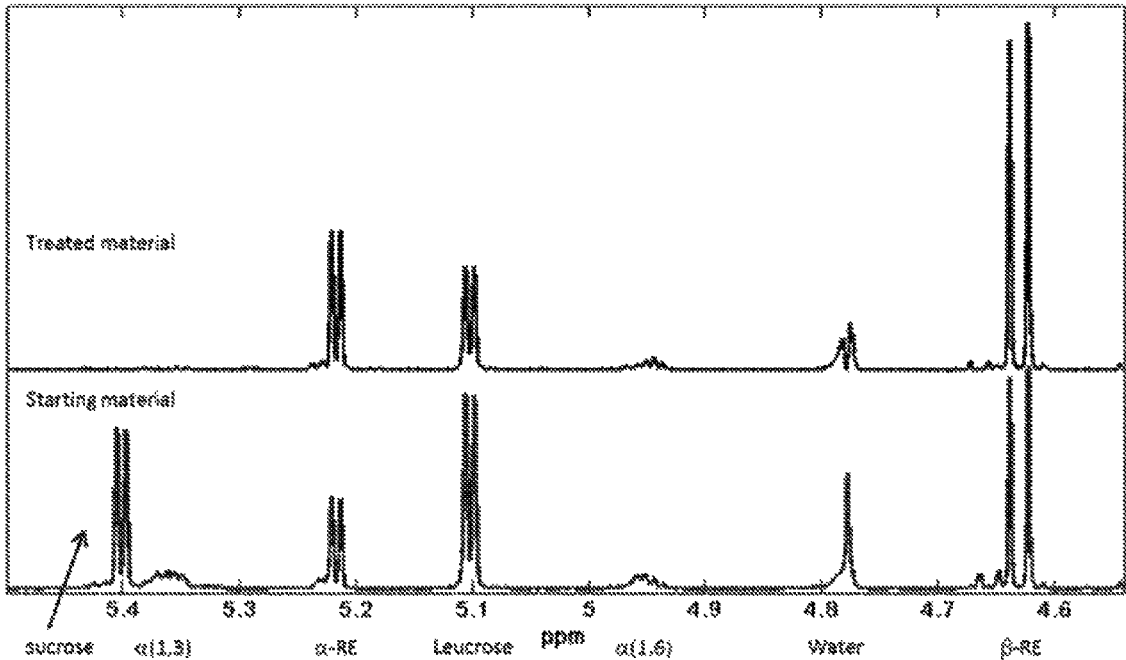


Figure 1

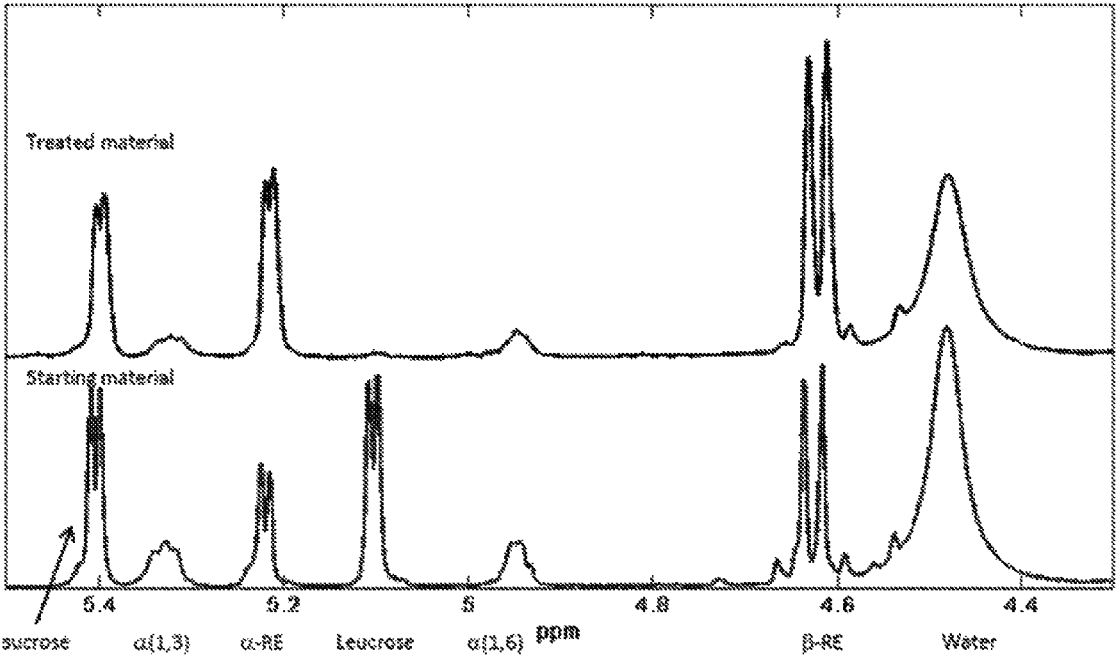


Figure 2

1

**ENZYMATIC HYDROLYSIS OF  
DISACCHARIDES AND  
OLIGOSACCHARIDES USING  
ALPHA-GLUCOSIDASE ENZYMES**

This application claims the benefit of U.S. Provisional Application Nos. 61/945,233 (filed Feb. 27, 2014), 61/945,241 (filed Feb. 27, 2014), 62/004,290 (filed May 29, 2014), 62/004,308 (filed May 29, 2014), 62/004,312 (filed May 29, 2014), 62/004,300 (filed May 29, 2014), 62/004,314 (filed May 29, 2014), and 62/004,305 (filed May 29, 2014), and of International Appl. No. PCT/CN2015/073269 (filed Feb. 25, 2015), all of which are incorporated herein by reference in their entireties.

FIELD OF INVENTION

The invention is in the field of enzymatic hydrolysis of small sugar polymers. Specifically, this invention pertains to hydrolyzing disaccharides and oligosaccharides comprising one or more alpha-1,3 or alpha-1,6 glucosyl-glucose linkages with an alpha-glucosidase enzyme.

REFERENCE TO SEQUENCE LISTING  
SUBMITTED ELECTRONICALLY

The official copy of the sequence listing is submitted electronically via EFS-Web as an ASCII formatted sequence listing with a file named CL6220USNP\_SequenceListing\_T25.txt created on Feb. 11, 2015, and having a size of 266 kilobytes and is filed concurrently with the specification. The sequence listing contained in this ASCII-formatted document is part of the specification and is herein incorporated by reference in its entirety.

BACKGROUND

Transglucosidases (EC.2.4.1.24, 1,4-alpha-glucan 6-alpha-glucosyltransferase) are D-glucosyltransferase enzymes that catalyze both hydrolytic and transfer reactions on incubation with alpha-D-gluco-oligosaccharides (1951, Pazur and French, *J. Amer. Chem. Soc.* 73:3536). Maltose is the most preferred substrate for transglucosylation reactions with this enzyme. Transfer occurs most frequently to HO-6, producing isomaltose from D-glucose, or panose (6-O-alpha-glucosyl maltose) from maltose. Transglucosidase can also transfer a glucosyl residue to the HO-2 or HO-3 of another D-glucosyl unit to form Kojibiose or Nigerose. This enzyme can further transfer a D-glucosyl unit back to HO-4 to reform maltose.

As a result of transglucosylation reactions with transglucosidase, malto-oligosaccharide residues are converted to isomalto-oligosaccharides (IMO) containing a higher proportion of glucosyl residues linked by alpha-D-1,6 glycosidic linkages from the non-reducing end. IMO sugars are used in many food and beverage formulations in Asia. Brier et al. (U.S. Patent Appl. Publ. No. 2003/0167929) disclosed using transglucosidase to produce IMO from barley wort.

Poulose et al. (U.S. Patent Appl. Publ. No. 2008/0229514) disclosed using transglucosidase to degrade polysaccharides such as xanthan and guar gums. Xanthan gum comprises a cellulosic backbone in which alternate glucoses are 1,3-linked to branches containing mannose and glucuronic acid. The backbone of guar gum comprises beta-1,4-linked mannose residues to which galactose residues are alpha-1,6-linked at every other mannose.

2

Lantero et al. (U.S. Pat. No. 5,770,437) disclosed using a transglucosidase to degrade sucrose, melezitose and trehalulose. These sugars comprise glucose linked to fructose via 1,2-(sucrose), 1,3-(melezitose), or 1,1-(trehalulose) linkages.

Although various hydrolytic activities of transglucosidase have been disclosed, this type of enzyme is generally considered to be an alpha-glucosidase, given its ability to hydrolyze alpha-linkages between two glucosyl residues. For example, transglucosidase is associated with having maltase activity (hydrolysis of the alpha-1,4 glycosidic link between the two glucosyl residues of maltose), which is a type of alpha-glucosidase activity.

Notwithstanding the foregoing disclosures, surprisingly, it has now been found that alpha-glucosidases such as transglucosidase (EC 2.4.1.24) can hydrolyze alpha-1,3 and alpha-1,6 glycosidic linkages of glucosyl-glucose. Alpha-glucosidase enzymes are disclosed herein as being useful for degrading disaccharides and oligosaccharides containing glucosyl-alpha-1,3-glucose and glucosyl-alpha-1,6-glucose.

SUMMARY OF INVENTION

In one embodiment, the invention concerns a method of hydrolyzing an alpha-1,3 or alpha-1,6 glucosyl-glucose linkage in a saccharide comprising at least one alpha-1,3 or alpha-1,6 glucosyl-glucose linkage, wherein the saccharide is a disaccharide or oligosaccharide, and wherein the method comprises: contacting the saccharide with an alpha-glucosidase enzyme under suitable conditions, wherein the alpha-glucosidase enzyme hydrolyzes at least one alpha-1,3 or alpha-1,6 glucosyl-glucose linkage of the saccharide, and wherein the amount of the saccharide is reduced compared to the amount of the saccharide that was present prior to the contacting step.

In another embodiment, the alpha-glucosidase enzyme of the hydrolysis method is immobilized.

In another embodiment, the saccharide of the hydrolysis method has a degree of polymerization before hydrolysis of 3 to 7. In another embodiment, the concentration of the saccharide after the contacting step is less than 50% of the concentration of the saccharide that was present prior to the contacting step.

In another embodiment, the suitable conditions of the hydrolysis method comprise (i) a glucan synthesis reaction, or (ii) a fraction obtained from the glucan synthesis reaction; wherein the saccharide is a byproduct of the glucan synthesis reaction. The glucan synthesis reaction produces at least one insoluble alpha-glucan product in another embodiment. In another embodiment, the fraction is a filtrate of the glucan synthesis reaction. In another embodiment, the glucan synthesis reaction produces at least one soluble alpha-glucan product that is (i) a product of a glucosyltransferase, or (ii) a product of the concerted action of both a glucosyltransferase and an alpha-glucanohydrolase capable of hydrolyzing glucan polymers having one or more alpha-1,3-glycosidic linkages or one or more alpha-1,6-glycosidic linkages. The fraction is a chromatographic fraction of the glucan synthesis reaction in another embodiment in which the glucan synthesis reaction produces at least one soluble alpha-glucan product.

In another embodiment, the alpha-glucosidase enzyme is a transglucosidase. In another embodiment, the transglucosidase comprises an amino acid sequence that is at least 90% identical to SEQ ID NO:1

In another embodiment, the invention concerns a composition produced by contacting a saccharide with an alpha-glucosidase enzyme, wherein the saccharide is a disaccharide or oligosaccharide and comprises at least one alpha-1,3 or alpha-1,6 glucosyl-glucose linkage, wherein the alpha-glucosidase enzyme hydrolyzes at least one alpha-1,3 or alpha-1,6 glucosyl-glucose linkage of the saccharide, and wherein the composition comprises a reduced amount of the saccharide compared to the amount of the saccharide that was present prior to the contacting step.

In another embodiment, the saccharide of the composition has a degree of polymerization before hydrolysis of 3 to 7. The concentration of the saccharide after the contacting step is less than 50% of the concentration of the saccharide that was present prior to the contacting step, for example.

In another embodiment, the saccharide of the composition is in (i) a glucan synthesis reaction, or (ii) a fraction obtained from the glucan synthesis reaction; wherein the saccharide is a byproduct of the glucan synthesis reaction. In another embodiment, the fraction is a filtrate of the glucan synthesis reaction or a chromatographic fraction of the glucan synthesis reaction.

In another embodiment, the invention concerns a method of enriching fructose present in a fraction of a glucan synthesis reaction, comprising: (a) contacting a fraction obtained from a glucan synthesis reaction with an alpha-glucosidase enzyme under suitable conditions, wherein the alpha-glucosidase enzyme hydrolyzes at least one alpha-1,3 or alpha-1,6 glucosyl-glucose linkage of a disaccharide or oligosaccharide comprised within the fraction; and (b) separating fructose from the hydrolyzed fraction of step (a) to obtain a composition having a higher concentration of fructose compared to the fructose concentration of the fraction of step (a).

In another embodiment, the invention concerns a fermentation method comprising: (a) contacting a fraction obtained from a glucan synthesis reaction with an alpha-glucosidase enzyme under suitable conditions, wherein the alpha-glucosidase enzyme hydrolyzes at least one alpha-1,3 or alpha-1,6 glucosyl-glucose linkage of a disaccharide or oligosaccharide comprised within the fraction; (b) fermenting the fraction of step (a) with a microbe to yield a product, wherein the fermenting can be performed after step (a) or simultaneously with step (a); and (c) optionally, isolating the product of (b); wherein the yield of the product of (b) is increased compared to the product yield of fermenting a fraction of the glucan synthesis reaction that has not been contacted with the enzyme.

#### BRIEF DESCRIPTION OF THE DRAWINGS AND SEQUENCES

FIG. 1: <sup>1</sup>H NMR spectra of glucan reaction filtrate material before (starting material) and after (treated material) hydrolysis treatment with NOVO 188 enzyme (see Examples 2-3).

FIG. 2: <sup>1</sup>H NMR spectra of glucan reaction filtrate material before (starting material) and after (treated material) hydrolysis treatment with TG L-2000 transglucosidase (see Examples 2-3).

TABLE 1

Summary of Nucleic Acid and Protein Sequence Identification Numbers		
Description	Nucleic acid SEQ ID NO.	Protein SEQ ID NO.
5 "TG L-2000", <i>A. niger</i> transglucosidase (mature form without signal peptide)		1 (965 aa)
10 "GC 321 Glucoamylase", <i>T. reesei</i> glucoamylase (TrGA) (mature form without signal peptide)		2 (599 aa)
"gtfJ", <i>Streptococcus salivarius</i> glucosyltransferase. The first 42 amino acids of the protein are deleted compared to GENBANK Identification No. 47527; a start methionine is included.		3 (1477 aa)
15 "Aclglu1", <i>Aspergillus clavatus</i> alpha-glucosidase, full-length precursor form including signal peptide.	4 (3147 bases)	5 (990 aa)
20 "Aclglu1", <i>Aspergillus clavatus</i> alpha-glucosidase, mature form lacking signal peptide.		6 (971 aa)
"Nfiglu1", <i>Neosartorya fischeri</i> alpha-glucosidase, full-length precursor form including signal peptide.	7 (3158 bases)	8 (988 aa)
25 "Nfiglu1", <i>Neosartorya fischeri</i> alpha-glucosidase, mature form lacking signal peptide.		9 (969 aa)
"Ncrglu1", <i>Neurospora crassa</i> alpha-glucosidase, full-length precursor form including signal peptide.	10 (3385 bases)	11 (1044 aa)
30 "Ncrglu1", <i>Neurospora crassa</i> alpha-glucosidase, mature form lacking signal peptide.		12 (1022 aa)
"TauSec098", <i>Rasamsonia composticola</i> alpha-glucosidase, full-length precursor form including signal peptide.	13 (3293 bases)	14 (1035 aa)
35 "TauSec098", <i>Rasamsonia composticola</i> alpha-glucosidase, mature form lacking signal peptide.		15 (1013 aa)
"TauSec099", <i>Rasamsonia composticola</i> alpha-glucosidase, full-length precursor form including signal peptide.	16 (3162 bases)	17 (990 aa)
40 "TauSec099", <i>Rasamsonia composticola</i> alpha-glucosidase, mature form lacking signal peptide.		18 (973 aa)
"BloGlu1", <i>Bifidobacterium longum</i> (subsp. longum JDM301) alpha-glucosidase (wild type).	19 (1815 bases)	20 (604 aa)
45 "BloGlu1", <i>Bifidobacterium longum</i> (subsp. longum JDM301) alpha-glucosidase, codon-optimized sequence.	21 (1812 bases)	
"BloGlu2", <i>Bifidobacterium longum</i> alpha-glucosidase (wild type).		22 (604 aa)
"BloGlu2", <i>Bifidobacterium longum</i> alpha-glucosidase, codon-optimized sequence encoding amino acid	23 (1812 bases)	24 (604 aa)
50 sequence.		
"BloGlu3", <i>Bifidobacterium longum</i> (subsp. F8) alpha-glucosidase (wild type)	25 (1815 bases)	26 (604 aa)
55 "BloGlu3", <i>Bifidobacterium longum</i> (subsp. F8) alpha-glucosidase, codon-optimized sequence encoding amino acid sequence.	27 (1812 bases)	
"BpsGlu1", <i>Bifidobacterium pseudolongum</i> alpha-glucosidase (wild type).		28 (585 aa)
60 "BpsGlu1", <i>Bifidobacterium pseudolongum</i> alpha-glucosidase, codon-optimized sequence encoding amino acid sequence.	29 (1755 bases)	30 (586 aa)
"BthGlu1", <i>Bifidobacterium thermophilum</i> RBL67 alpha-glucosidase (wild type).	31 (1806 bases)	32 (601 aa)
65 "BthGlu1", <i>Bifidobacterium thermophilum</i> RBL67 alpha-glucosidase, codon-optimized sequence.	33 (1803 bases)	

5

TABLE 1-continued

Summary of Nucleic Acid and Protein Sequence Identification Numbers		
Description	Nucleic acid SEQ ID NO.	Protein SEQ ID NO.
"BbrGlu2", <i>Bifidobacterium breve</i> alpha-glucosidase (wild type).		34 (662 aa)
"BbrGlu2", <i>Bifidobacterium breve</i> alpha-glucosidase, codon-optimized sequence encoding amino acid sequence.	35 (1812 bases)	36 (604 aa)
"BbrGlu5", <i>Bifidobacterium breve</i> ACS-071-V-Sch8b alpha-glucosidase (wild type).	37 (1821 bases)	38 (606 aa)
"BbrGlu5", <i>Bifidobacterium breve</i> ACS-071-V-Sch8b alpha-glucosidase, codon-optimized sequence.	39 (1818 bases)	
"Gtf-S", <i>Streptococcus</i> sp. C150 glucosyltransferase, GENBANK GI No. 321278321.		40 (1570 aa)
"GTF0459", <i>Streptococcus</i> sp. C150 glucosyltransferase, N-terminal-truncated version of GENBANK GI No. 321278321.	41 (4179 bases)	42 (1392 aa)
"Gtf-C", <i>Streptococcus mutans</i> MT-4239 glucosyltransferase, GENBANK GI No. 3130088.		43 (1455 aa)
"GTF0088BsT1", <i>Streptococcus mutans</i> MT-4239 glucosyltransferase, N- and C-terminal-truncated version of GENBANK GI No. 3130088.	44 (2715 bases)	45 (904 aa)
"MUT3325", <i>Penicillium marneffeii</i> ATCC 18224 mutanase, GENBANK GI No. 212533325.	46 (1308 bases)	47 (435 aa)

#### DETAILED DESCRIPTION OF THE INVENTION

The disclosures of all cited patent and non-patent literature are incorporated herein by reference in their entirety.

As used herein, the term "invention" or "disclosed invention" is not meant to be limiting, but applies generally to any of the inventions defined in the claims or described herein. These terms are used interchangeably herein.

The terms "saccharide", "saccharide molecule" and "carbohydrate" are used interchangeably herein and refer to a disaccharide or oligosaccharide, unless otherwise noted. A "disaccharide" herein refers to a carbohydrate having two monosaccharides joined by a glycosidic linkage. An "oligosaccharide" herein refers to a carbohydrate that consists of 2 to 9 monosaccharides, for example, joined by glycosidic linkages. An oligosaccharide can also be referred to herein as an "oligomer". Monosaccharides that are comprised within a disaccharide or oligosaccharide can be referred to as "monosaccharide units" or "monomeric units", for example. Preferred monosaccharides herein are fructose and glucose.

The terms "glycosidic linkage" and "glycosidic bond" are used interchangeably herein and refer to the type of covalent bond that joins a carbohydrate molecule to another carbohydrate molecule.

The terms "alpha-1,3 glucosyl-glucose linkage", "alpha-1,3 glucose-glucose linkage" and "glucose-alpha 1,3-glucose" herein refers to an alpha-1,3-glycosidic linkage between two alpha-D-glucose molecules. The terms "alpha-1,6 glucosyl-glucose linkage", "alpha-1,6 glucose-glucose linkage" and "glucose-alpha 1,6-glucose" herein refers to an alpha-1,6-glycosidic linkage between two alpha-D-glucose molecules. Alpha-1,3 glucosyl-glucose linkage(s) and/or

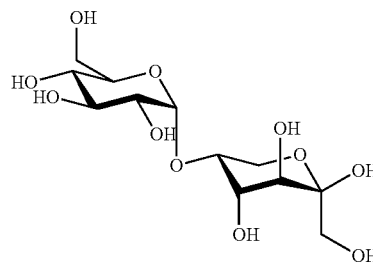
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alpha-1,6 glucosyl-glucose linkage(s) herein are comprised within a disaccharide or oligosaccharide in certain embodiments.

The terms "alpha-1,5 glucosyl-fructose linkage", "alpha-1,5 glucose-fructose linkage" and "glucose-alpha-1,5-fructose" herein refers to an alpha-1,5-glycosidic linkage between an alpha-D-glucose molecule and a fructose molecule. An alpha-1,5 glucosyl-fructose linkage herein is comprised within a disaccharide or oligosaccharide in certain embodiments.

"Alpha-D-glucose" herein can also be referred to as "glucose".

A disaccharide containing an alpha-1,5 glucosyl-fructose linkage is referred to herein as leucrose. The terms "leucrose" and "D-glucopyranosyl-alpha(1-5)-D-fructopyranose" are used interchangeably herein. Leucrose has the following structure:



The terms "alpha-glucosidase", "alpha-1,4-glucosidase", and "alpha-D-glucoside glucohydrolase" are used interchangeably herein. Alpha-glucosidases (EC 3.2.1.20) ("EC" refers to Enzyme Commission number) have previously been recognized as enzymes that catalyze hydrolytic release of terminal, non-reducing (1,4)-linked alpha-D-glucose residues from oligosaccharide (e.g., disaccharide) and polysaccharide substrates. Alpha-glucosidases are now disclosed herein to also have hydrolytic activity toward alpha-1,5 glucosyl-fructose linkages, and hydrolytic activity toward alpha-1,3 and alpha-1,6 glucosyl-glucose linkages. Transglucosidase and glucoamylase enzymes are examples of alpha-glucosidases with such activity.

The terms "transglucosidase" (TG), "transglucosidase enzyme", and "1,4-alpha-glucan 6-alpha-glucosyltransferase" are used interchangeably herein. Transglucosidases (EC 2.4.1.24) have previously been recognized as D-glucosyltransferase enzymes that catalyze both hydrolytic and transfer reactions on incubation with certain alpha-D-glucoligosaccharides. Transglucosidases are now disclosed herein to also have hydrolytic activity toward alpha-1,5 glucosyl-fructose linkages, and hydrolytic activity toward alpha-1,3 and alpha-1,6 glucosyl-glucose linkages.

The terms "glucoamylase" (GA), "glucoamylase enzyme", and "alpha-1,4-glucan glucohydrolase" are used interchangeably herein. Glucoamylases (EC 3.2.1.3) have previously been recognized as exo-acting enzymes that catalyze hydrolysis of both alpha-1,4 and alpha-1,6 glycosidic linkages from non-reducing ends of glucose-containing di-, oligo- and poly-saccharides. Glucoamylases are now disclosed herein to also have hydrolytic activity toward alpha-1,5 glucosyl-fructose linkages.

Enzymatic hydrolysis is a process in which an enzyme facilitates the cleavage of bonds in molecules with the addition of the elements of water. "Hydrolyzing", "hydrolysis of", or "hydrolytic activity toward" an alpha-1,3 or

alpha 1,6 glucosyl-glucose linkage herein refers to enzymatic hydrolysis of the alpha-1,3 or alpha-1,6 glycosidic linkage between two glucose molecules by an alpha-glucosidase such as a transglucosidase. Such hydrolysis occurs when contacting a disaccharide or oligosaccharide containing an alpha-1,3 and/or alpha-1,6 glucosyl-glucose linkage with an alpha-glucosidase herein under suitable conditions. Thus, a “hydrolysis reaction” herein comprises at least (i) a disaccharide or oligosaccharide containing an alpha-1,3 and/or alpha-1,6 glucosyl-glucose linkage(s), and (ii) an alpha-glucosidase.

The term “saccharification” herein refers to a process of breaking a saccharide (disaccharide or oligosaccharide) into its monosaccharide components. A saccharide can be saccharified in a hydrolysis reaction herein.

“Suitable conditions” for contacting a saccharide (disaccharide or oligosaccharide) comprising at least one alpha-1,3 and/or alpha-1,6 glucosyl-glucose linkage with an alpha-glucosidase herein refer to those conditions (e.g., temperature, pH, time) that support the hydrolysis of one or more alpha-1,3 and/or alpha-1,6 glucosyl-glucose linkages in the saccharide by the alpha-glucosidase. Suitable conditions can comprise “aqueous conditions”, for example, comprising at least 20 wt % water. Aqueous conditions may characterize a solution or mixture. The solution or mixture in which a saccharide comprising at least one alpha-1,3 and/or alpha-1,6 glucosyl-glucose linkage is contacted with an alpha-glucosidase can be referred to as an alpha-glucosidase reaction, for example (e.g., a transglucosidase or glucoamylase reaction).

An “immobilized” enzyme herein refers to an enzyme that is attached to an inert, insoluble material. Methods for preparing immobilized enzymes are disclosed, for example, in U.S. Pat. No. 5,541,097, which is incorporated herein by reference.

The terms “glucan” and “glucan polymer” are used interchangeably herein and refer to a polysaccharide of glucose monomers linked by glycosidic bonds. An “alpha-glucan” herein refers to a glucan polymer comprising at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% alpha-glycosidic linkages.

An “insoluble glucan” herein refers to a glucan polymer that is not soluble in aqueous conditions. An example of insoluble glucan herein is poly alpha-1,3-glucan with a DP of at least 8 or 9. A glucosyltransferase reaction in certain embodiments as presently disclosed produces at least one insoluble glucan product.

The terms “soluble glucan”, “soluble alpha-glucan”, “soluble fiber”, “soluble glucan fiber”, “soluble dietary fiber” and the like are used interchangeably herein to refer to a glucan polymer that is soluble in aqueous conditions. Examples of soluble glucan herein are certain oligosaccharides, such as poly alpha-1,3-glucan with a DP less than 8, and certain oligosaccharides disclosed in the Examples provided below. A glucosyltransferase reaction in certain embodiments as presently disclosed produces at least one soluble glucan product. Another set of features that characterizes soluble alpha-glucan compounds in certain embodiments herein is that they are (i) water-soluble glucose oligomers having a degree of polymerization of 3 or more, (ii) digestion-resistant (i.e., exhibit very slow or no digestibility) with little or no absorption in the human small intestine, and (iii) at least partially fermentable in the lower gastrointestinal tract. Digestibility of a soluble glucan fiber composition can be measured using AOAC method 2009.01, for example.

The terms “poly alpha-1,3-glucan” and “alpha-1,3-glucan polymer” are used interchangeably herein. Poly alpha-1,3-glucan is a polymer comprising glucose monomeric units linked together by glycosidic linkages, wherein at least about 50% of the glycosidic linkages are alpha-1,3-glycosidic linkages. The term “alpha-1,3-glycosidic linkage” as used herein refers to the type of covalent bond that joins alpha-D-glucose molecules to each other through carbons 1 and 3 on adjacent alpha-D-glucose rings.

The “molecular weight” of a glucan herein can be represented as number-average molecular weight ( $M_n$ ) or as weight-average molecular weight ( $M_w$ ). Alternatively, molecular weight can be represented as Daltons, grams/mole,  $DP_w$  (weight average degree of polymerization), or  $DP_n$  (number average degree of polymerization). Various means are known in the art for calculating these molecular weight measurements such as with high-pressure liquid chromatography (HPLC), size exclusion chromatography (SEC), or gel permeation chromatography (GPC).

The terms “glucosyltransferase enzyme”, “gtf enzyme”, “gtf enzyme catalyst”, “gtf”, “glucansucrase” and the like are used interchangeably herein. The activity of a gtf enzyme herein catalyzes the reaction of sucrose substrate to make the products glucan and fructose. Other products (byproducts) of a gtf reaction can include glucose (results from when glucose is hydrolyzed from the glucosyl-gtf enzyme intermediate complex), various soluble oligosaccharides (e.g., DP2-DP7), and leucrose (results from when glucose of the glucosyl-gtf enzyme intermediate complex is linked to fructose). Wild type forms of glucosyltransferase enzymes generally contain (in the N-terminal to C-terminal direction) a signal peptide, a variable domain, a catalytic domain, and a glucan-binding domain. A glucosyltransferase herein is classified under the glycoside hydrolase family 70 (GH70) according to the CAZy (Carbohydrate-Active EnZymes) database (Cantarel et al., *Nucleic Acids Res.* 37:D233-238, 2009).

The term “sucrose” herein refers to a non-reducing disaccharide composed of an alpha-D-glucose molecule and a beta-D-fructose molecule linked by an alpha-1,2-glycosidic bond. Sucrose is known commonly as table sugar.

The terms “glucan synthesis reaction”, “glucan reaction” “gtf reaction” and the like are used interchangeably herein and refer to a reaction that is performed by a glucosyltransferase enzyme. A glucan synthesis reaction as used herein generally refers to a solution comprising at least one active glucosyltransferase enzyme in a solution comprising sucrose and water, and optionally other components. Other components that can be in a glucan synthesis reaction herein include fructose, glucose, leucrose, soluble oligosaccharides (e.g., DP2-DP7), and soluble glucan product(s), for example. Also, one or more alpha-glucanohydrolase enzymes can be comprised in a glucan synthesis reaction in some aspects. It would be understood that certain glucan products, such as poly alpha-1,3-glucan with a degree of polymerization (DP) of at least 8 or 9, are water-insoluble and thus are not dissolved in a glucan synthesis reaction, but rather may be present out of solution.

The terms “alpha-glucanohydrolase” and “glucanohydrolase” are used interchangeably herein and refer to an enzyme capable of hydrolyzing an alpha-glucan oligomer. An alpha-glucanohydrolase can be defined by its endohydrolysis activity towards certain alpha-D-glycosidic linkages. Examples of alpha-glucanohydrolase enzymes herein include dextranases (EC 3.2.1.11; capable of endohydrolyzing alpha-1,6-linked glycosidic bonds), mutanases (EC 3.2.1.59; capable of endohydrolyzing alpha-1,3-linked gly-

cosidic bonds), and alternanases (EC 3.2.1.-; capable of endohydrolytically cleaving alternan).

Various factors including, but not limited to, level of branching, the type of branching, and the relative branch length within certain alpha-glucans may adversely impact the ability of an alpha-glucanohydrolase to endohydrolyze some glycosidic linkages.

The "percent dry solids" of a glucan synthesis reaction refers to the wt % of all the sugars in a glucan synthesis reaction. The percent dry solids of a gtf reaction can be calculated, for example, based on the amount of sucrose used to prepare the reaction.

A "fraction" of a glucan synthesis reaction herein refers to a liquid solution portion of a glucan synthesis reaction. A fraction can be a portion of, or all of, the liquid solution from a glucan synthesis reaction, and has been separated from a soluble or insoluble glucan product synthesized in the reaction. A fraction can optionally be referred to as a "mother liquor" in embodiments in which the product is an insoluble (solid) glucan product. An example of a fraction is a filtrate of a glucan synthesis reaction. Since a fraction can contain dissolved sugars such as sucrose, fructose, glucose, leucrose, soluble oligosaccharides (e.g., DP2-DP7), a fraction can also be referred to as a "mixed sugar solution" derived from a glucan synthesis reaction. A "hydrolyzed fraction" herein refers to a fraction that has been treated with an alpha-glucosidase herein to hydrolyze leucrose and/or oligosaccharides present in the fraction.

The terms "filtrate", "glucan reaction filtrate", "glucan filtrate" and the like are used interchangeably herein and refer to a fraction that has been filtered away from a solid glucan product synthesized in a glucan synthesis reaction. A "hydrolyzed filtrate" herein refers to a filtrate that has been treated with an alpha-glucosidase herein to hydrolyze leucrose and/or oligosaccharides present in the filtrate.

The terms "percent by volume", "volume percent", "vol %", "v/v %" and the like are used interchangeably herein. The percent by volume of a solute in a solution can be determined using the formula: [(volume of solute)/(volume of solution)] $\times$ 100%.

The terms "percent by weight", "weight percentage (wt %)", "weight-weight percentage (% w/w)" and the like are used interchangeably herein. Percent by weight refers to the percentage of a material on a mass basis as it is comprised in a composition, mixture, or solution. All percentages herein are weight percentages, unless otherwise noted.

As used herein, "polydispersity index", "PDI", "heterogeneity index", "dispersity" and the like refer to a measure of the distribution of molecular mass in a given polymer (e.g., a glucose oligomer such as a soluble alpha-glucan) sample and can be calculated by dividing the weight average molecular weight by the number average molecular weight ( $PDI = M_w/M_n$ ).

The terms "increased", "enhanced" and "improved" are used interchangeably herein. These terms refer to a greater quantity or activity such as a quantity or activity slightly greater than the original quantity or activity, or a quantity or activity in large excess compared to the original quantity or activity, and including all quantities or activities in between. Alternatively, these terms may refer to, for example, a quantity or activity that is at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19% or 20% more than the quantity or activity for which the increased quantity or activity is being compared.

The terms "sequence identity" or "identity" as used herein with respect to polynucleotide or polypeptide sequences refer to the nucleic acid bases or amino acid residues in two

sequences that are the same when aligned for maximum correspondence over a specified comparison window. Thus, "percentage of sequence identity" or "percent identity" refers to the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the results by 100 to yield the percentage of sequence identity.

The Basic Local Alignment Search Tool (BLAST) algorithm, which is available online at the National Center for Biotechnology Information (NCBI) website, may be used, for example, to measure percent identity between or among two or more of the polynucleotide sequences (BLASTN algorithm) or polypeptide sequences (BLASTP algorithm) disclosed herein. Alternatively, percent identity between sequences may be performed using a Clustal algorithm (e.g., ClustalW or ClustalV). For multiple alignments using a Clustal method of alignment, the default values may correspond to GAP PENALTY=10 and GAP LENGTH PENALTY=10. Default parameters for pairwise alignments and calculation of percent identity of protein sequences using a Clustal method may be KTUPLE=1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. For nucleic acids, these parameters may be KTUPLE=2, GAP PENALTY=5, WINDOW=4 and DIAGONALS SAVED=4. Alternatively still, percent identity between sequences may be performed using an EMBOSS algorithm (e.g., needle) with parameters such as GAP OPEN=10, GAP EXTEND=0.5, END GAP PENALTY=false, END GAP OPEN=10, END GAP EXTEND=0.5 using a BLOSUM matrix (e.g., BLOSUM62).

Various polypeptide amino acid sequences are disclosed herein as features of certain embodiments. Variants of these sequences that are at least about 70-85%, 85-90%, or 90%-95% identical to the sequences disclosed herein can be used. Alternatively, a variant amino acid sequence can have at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity with a sequence disclosed herein. A variant amino acid sequence herein has the same function/activity of the disclosed sequence, or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the function/activity of a disclosed sequence.

The term "isolated" as used in certain embodiments refers to any cellular component that is completely separated from its native source (e.g., an isolated polynucleotide or polypeptide molecule). In some instances, an isolated polynucleotide or polypeptide molecule is part of a greater composition, buffer system or reagent mix. For example, the isolated polynucleotide or polypeptide molecule can be comprised within a cell or organism in a heterologous manner. Another example is an isolated alpha-glucosidase (e.g., glucoamylase, transglucosidase), or glucosyltransferase enzyme. The enzyme reactions (e.g., alpha-glucosidase reaction, glucosyltransferase reaction) disclosed herein are synthetic, non-naturally occurring processes.

Embodiments of the disclosed invention concern a method of hydrolyzing an alpha-1,3 or alpha-1,6 glucosyl-glucose linkage in a saccharide comprising at least one alpha-1,3 or alpha-1,6 glucosyl-glucose linkage. The saccharide is a disaccharide or oligosaccharide. This method comprises contacting the saccharide with an alpha-glucosidase enzyme under suitable conditions. In the contacting step, the alpha-glucosidase enzyme hydrolyzes at least one alpha-1,3 or alpha-1,6 glucosyl-glucose linkage of the saccharide. Due to this hydrolysis, the amount of the saccharide is reduced compared to the amount of the saccharide that was present prior to the contacting step. Thus, this hydrolysis method can alternatively be referred to as a method of reducing the amount of a saccharide in a composition.

Significantly, it is believed to be previously unknown that alpha-glucosidase enzymes can hydrolyze alpha-1,3 and alpha-1,6 glucosyl-glucose linkages. Alpha-glucosidase reactions following this hydrolysis method can thus be used to remove oligosaccharide byproducts containing these glucose-glucose linkages from a glucan synthesis reaction and/or a fraction obtained therefrom.

Such removal represents an improvement over chemical processes of byproduct removal, such as acid hydrolysis, which can result in degradation of glucan product. Finally, a glucan reaction fraction that is treated according to the above hydrolysis method is better-suited for downstream applications such as fermentation, for example, since the level of glucose monosaccharides is increased in the fraction. Monosaccharides are generally more tractable for downstream processes compared to oligosaccharide byproducts.

An alpha-glucosidase (EC 3.2.1.20) is used in embodiments herein to hydrolyze alpha-1,3 and/or alpha-1,6 glucosyl-glucose linkages in a saccharide comprising at least one of these linkages. Alpha-glucosidase enzymes have previously been recognized to catalyze hydrolytic release of terminal, non-reducing (1,4)-linked alpha-D-glucose residues from oligosaccharide (e.g., disaccharide) and polysaccharide substrates. These enzymes are now disclosed herein to also have hydrolytic activity toward alpha-1,3 and alpha-1,6 glucosyl-glucose linkages, for example.

An alpha-glucosidase can be from any source (e.g., plant, animal, microbe such as a bacteria or fungus/yeast), for example, such as those sources disclosed below from which a transglucosidase and/or glucoamylase can be derived. For example, an alpha-glucosidase can be a fungal alpha-glucosidase. Other examples of suitable alpha-glucosidases herein include those disclosed in U.S. Pat. Nos. 6,355,467, 5,922,580, 5,795,766, 5,763,252, and 8,633,006, which are all incorporated herein by reference.

An alpha-glucosidase enzyme in certain embodiments herein may comprise the amino acid sequence of SEQ ID NO:5, 6, 8, 9, 11, 12, 14, 15, 17, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, or that of DIAZYME RDF ULTRA (DuPont Industrial Biosciences). Alternatively, an alpha-glucosidase enzyme may comprise an amino acid sequence that is at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:5, 6, 8, 9, 11, 12, 14, 15, 17, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, or to the amino acid sequence of DIAZYME RDF ULTRA, and have hydrolytic activity toward alpha-1,3 and/or alpha-1,6 glucosyl-glucose linkages in saccharides. Several of the foregoing sequences, for example, are mature alpha-glucosidases that lack an N-terminal signal peptide. For such sequences, it would be understood that an N-terminal start-methionine would typically be added (if necessary) (directly or via an intervening heterologous amino acid sequence such as an epitope) if

expressing it without using a signal peptide (such as with an expression system where the enzyme is expressed intracellularly and obtained from a cell lysate).

A transglucosidase (EC 2.4.1.24; 1,4-alpha-glucan 6-alpha-glucosyltransferase) can be used in certain embodiments herein as an alpha-glucosidase to hydrolyze alpha-1,3 and/or alpha-1,6 glucosyl-glucose linkages in a saccharide comprising at least one of these linkages. This class of enzymes has previously been recognized as D-glucosyltransferase enzymes that catalyze hydrolytic and transfer reactions on incubation with certain alpha-D-glucosaccharides. Transglucosidases as now disclosed herein also have hydrolytic activity toward alpha-1,3 and alpha-1,6 glucosyl-glucose linkages.

A transglucosidase enzyme herein may be derived from any microbial source, such as a bacteria or fungus. Examples of fungal transglucosidases include, but are not limited to, those of *Trichoderma* species (e.g., *T. reesei*), *Aspergillus* species and *Neosartorya* species (e.g., *N. fischeri*). Examples of *Aspergillus* species from which a transglucosidase may be derived include, but are not limited to, *A. niger*, *A. awamori*, *A. oryzae*, *A. terreus*, *A. clavatus*, *A. fumigatus* and *A. nidulans*. Other examples of transglucosidase enzymes useful herein are described in Barker et al. (1953, *J. Chem. Soc.* 3588-3593); Pazur et al. (1986, *Carbohydr. Res.* 149:137-147), Nakamura et al. (1997, *J. Biotechnol.* 53:75-84), and U.S. Patent Appl. Publ. No. 2008/0229514, all of which are incorporated herein by reference. Still other examples of transglucosidase enzymes useful herein are those that are thermostable; U.S. Pat. No. 4,689,296, which is incorporated herein by reference, discloses a process for producing thermostable transglucosidase. Yet more examples of transglucosidase enzymes useful herein may be any of those in the GENBANK database (NCBI), such as accession numbers: D45356 (GID:2645159, *A. niger*), BAD06006.1 (GID:4031328, *A. awamori*), BAA08125.1 (GID:1054565, *A. oryzae*), XP\_001210809.1 (GID:115492363, *A. terreus*), XP\_001216899.1 (GID:115433524, *A. terreus*), XP\_001271891.1 (GID:121707620, *A. clavatus*), XP\_751811.1 (GID:70993928, *A. fumigatus*), XP\_659621.1 (GID:67523121, *A. nidulans*), XP\_001266999.1 (GID:119500484, *N. fischeri*) and XP\_001258585.1 (GID:119473371, *N. fischeri*), which are all incorporated herein by reference. Alternatively, a transglucosidase herein may have an amino acid sequence that is at least 90% or 95% identical with the amino acid sequence of any of the foregoing disclosed transglucosidase sequences, and have hydrolytic activity toward alpha-1,3 and/or alpha-1,6 glucosyl-glucose linkages in saccharides. All of the foregoing transglucosidases, when used in a hydrolysis reaction herein, are preferably in a mature form lacking an N-terminal signal peptide.

A transglucosidase enzyme in certain embodiments herein may comprise the amino acid sequence of SEQ ID NO:1 (TG L-2000), which is an *A. niger* transglucosidase (U.S. Patent Appl. Publ. No. 2008/0229514). Alternatively, a transglucosidase may comprise an amino acid sequence that is at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:1 and have hydrolytic activity toward alpha-1,3 and/or alpha-1,6 glucosyl-glucose linkages in saccharides. Any of SEQ ID NO:1 or variants thereof can be produced following the disclosure of U.S. Patent Appl. Publ. No. 2008/0229514, for example, which is incorporated herein by reference. SEQ ID NO:1 is a mature transglucosidase that lacks an N-terminal signal peptide. Since SEQ ID NO:1 does not begin with a methionine residue, it would be understood that an N-terminal

start-methionine would typically be added to SEQ ID NO:1 (directly or via an intervening heterologous amino acid sequence such as an epitope) if expressing it without using a signal peptide (such as with an expression system where the enzyme is expressed intracellularly and obtained from a cell lysate).

A glucoamylase (EC 3.2.1.3; alpha-1,4-glucan glucohydrolase) can be used in certain embodiments herein as an alpha-glucosidase. For example, a glucoamylase can be included with a transglucosidase in each of the hydrolysis reaction settings/conditions disclosed herein. In this context, a glucoamylase can be used to hydrolyze (i) an alpha-1,5 glucosyl-fructose linkage, and/or (ii) an alpha-1,3 and/or alpha-1,6 glucosyl-glucose linkage present in a saccharide containing any of these linkage types. This class of enzymes has previously been recognized as exo-acting enzymes that catalyze hydrolysis of both alpha-1,4 and alpha-1,6 glycosidic linkages from non-reducing ends of glucose-containing di-, oligo- and poly-saccharides. Glucoamylases as now disclosed herein also have hydrolytic activity toward alpha-1,5 glucosyl-fructose linkages. In certain embodiments, an alpha-glucosidase is not a glucoamylase.

A glucoamylase enzyme herein may be derived from any microbial source, such as a bacteria or fungus. Examples of bacterial glucoamylases include, but are not limited to, those of *Bacillus* species (e.g., *B. alkalophilus*, *B. amyloliquefaciens*, *B. lentus*, *B. licheniformis*, *B. stearothermophilus*, *B. subtilis*, *B. thuringiensis*) and *Streptomyces* species (e.g., *S. lividans*). Examples of fungal glucoamylases include, but are not limited to, those of *Trichoderma* species (e.g., *T. reesei*, *T. longibrachiatum*, *T. strictipilis*, *T. asperellum*, *T. konilangbra*, *T. hazianum*), *Aspergillus* species (e.g., *A. niger*, *A. oryzae*, *A. terreus*, *A. clavatus*, *A. nidulans*, *A. kawachi*, *A. awamon*), *Rhizopus* species (e.g., *R. oryzae*, *R. niveus*), *Talaromyces* species (e.g., *T. emersonii*, *T. thermophilus*, *T. dupontii*), *Mucor* species, *Hypocrea* species (e.g., *H. gelatinosa*, *H. orientalis*, *H. vinosa*, *H. citrina*), *Fusarium* species (e.g., *F. oxysporum*, *F. roseum*, *F. venenatum*), *Neurospora* species (e.g., *N. crassa*), *Humicola* species (e.g., *H. grisea*, *H. insolens*, *H. lanuginosa*), *Penicillium* species (e.g., *P. notatum*, *P. chrysogenum*) and *Saccharomyces* species (e.g., *S. fibuligera*).

Examples of these bacterial and fungal glucoamylases for use herein are disclosed in U.S. Pat. Appl. Publ. No. 2013/0102035, which is incorporated herein by reference. Other examples of glucoamylase enzymes useful herein are described in Svensson et al. (1983, *Carlsberg Res. Commun.* 48:529-544), Boel et al. (1984, *EMBO J.* 3:1097-1102); Hayashida et al. (1989, *Agric. Biol. Chem.* 53:923-929); U.S. Pat. Nos. 5,024,941, 4,794,175, 4,247,637, 6,255,084, 6,620,924, Ashikari et al. (1986, *Agric. Biol. Chem.* 50:957-964), Ashikari et al. (1989, *Appl. Microbiol. Biotechnol.* 32:129-133), U.S. Pat. Nos. 4,863,864; 4,618,579, Houghton-Larsen et al. (2003, *Appl. Microbiol. Biotechnol.* 62:210-217) and U.S. Pat. No. 7,413,887, all of which are incorporated herein by reference. Alternatively, a glucoamylase herein may have an amino acid sequence that is at least 90% or 95% identical with the amino acid sequence of any of the foregoing disclosed glucoamylase sequences, and have hydrolytic activity toward (i) alpha-1,5 glucosyl-fructose linkages and/or (ii) alpha-1,3 and/or alpha-1,6 glucosyl-glucose linkages. All of the foregoing glucoamylases, when used in a hydrolysis reaction herein, are preferably in a mature form lacking an N-terminal signal peptide. Commercially available glucoamylases useful herein include OPTI-

DEX L-400, GC 147, GC 321, G ZYME G990 4X, OPTI-MAX 7525, DEXTROZYME, DISTILLASE and GLUCZYME, for example.

A glucoamylase enzyme in certain embodiments herein may comprise the amino acid sequence of SEQ ID NO:2 (GC 321), which is a *T. reesei* glucoamylase. Alternatively, a glucoamylase may comprise an amino acid sequence that is at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:2 and have hydrolytic activity toward (i) alpha-1,5 glucosyl-fructose linkages and/or (ii) alpha-1,3 and/or alpha-1,6 glucosyl-glucose linkages. Any of SEQ ID NO:2 or variants thereof can be produced following the disclosures of U.S. Pat. No. 7,413,887 or U.S. Pat. Appl. Publ. No. 2013/0102035, for example, which are incorporated herein by reference. SEQ ID NO:2 is a mature glucoamylase that lacks an N-terminal signal peptide. Since SEQ ID NO:2 does not begin with a methionine residue, it would be understood that an N-terminal start-methionine would typically be added to SEQ ID NO:2 (directly or via an intervening heterologous amino acid sequence such as an epitope) if expressing it without using a signal peptide (such as with an expression system where the enzyme is expressed intracellularly and obtained from a cell lysate).

An alpha-glucosidase enzyme herein such as a transglucosidase or glucoamylase may be from a commercial source (e.g., DuPont Industrial Biosciences/Genencor, USA; Megazyme International, Ireland; Amano Enzyme Inc., Japan). Alternatively, such an enzyme may be produced by any means known in the art, such as described in U.S. Pat. Appl. Publ. No. 2008/0229514, U.S. Pat. No. 7,413,887 or U.S. Pat. Appl. Publ. No. 2013/0102035, which are incorporated herein by reference. For example, an alpha-glucosidase may be produced recombinantly in a heterologous expression system, such as a microbial or fungal heterologous expression system. Examples of heterologous expression systems include bacterial (e.g., *E. coli*, *Bacillus* sp.) and eukaryotic systems. Eukaryotic systems can employ yeast (e.g., *Pichia* sp., *Saccharomyces* sp.) or fungal (e.g., *Trichoderma* sp. such as *T. reesei*; *Aspergillus* species such as *A. niger*) expression systems, for example. The transglucosidase of SEQ ID NO:1 and glucoamylase of SEQ ID NO:2, and variants thereof, can be expressed in a *T. reesei* host, for example.

An alpha-glucosidase enzyme when used in a hydrolysis reaction herein is preferably in a mature form lacking an N-terminal signal peptide. An expression system for producing a mature alpha-glucosidase enzyme herein may employ an enzyme-encoding polynucleotide that further comprises sequence encoding an N-terminal signal peptide to direct extra-cellular secretion. The signal peptide in such embodiments is cleaved from the enzyme during the secretion process. The signal peptide may either be native or heterologous to the transglucosidase or glucoamylase. Alternatively, an alpha-glucosidase enzyme in a mature form can be provided by expressing it without using a signal peptide, such as with an expression system where the enzyme is expressed intracellularly and obtained from a cell lysate. In either scenario (secretion or intracellularly expressed), a heterologous amino acid sequence such as an epitope can optionally be included at the N-terminus of the alpha-glucosidase.

An alpha-glucosidase enzyme in certain embodiments may be provided in a hydrolysis reaction herein by direct use of a cell that expresses the enzyme(s). In other words, an alpha-glucosidase that is contacted with a saccharide can be present by virtue of its expression from a cell placed in the

suitable conditions for hydrolysis. Such a cell could thus be used in place of adding an isolated alpha-glucosidase preparation to the hydrolysis reaction. A cell for this purpose can be a bacterial, yeast, or fungal cell, for example. Examples of yeast include those from the genera *Saccharomyces* (e.g., *S. cerevisiae*), *Kluyveromyces*, *Candida*, *Pichia*, *Schizosaccharomyces*, *Hansenula*, *Kloeckera*, and *Schwanniomyces*. Other expression systems useful herein are disclosed in U.S. Patent. Appl. Publ. No. 2013/0323822, which is incorporated herein by reference.

A saccharide herein comprises at least one alpha-1,3 or alpha-1,6 glucosyl-glucose linkage. Thus, depending on the length of the saccharide, it may contain 1, 2, 3, 4, 5, 6, 7, or 8 alpha-1,5 glucosyl-glucose linkages, for example. A saccharide preferably contains 1, 2, or 3 linkages of this type. A saccharide in other preferred embodiments only has alpha-1,3 and/or alpha-1,6 glucosyl-glucose linkages. In other embodiments, a saccharide can have one or more alpha-1,5 glucosyl-fructose linkages.

Since a saccharide herein comprises at least one alpha-1,3 or alpha-1,6 glucosyl-glucose linkage, the saccharide comprises at least two glucose units. In certain embodiments, a saccharide herein comprises only glucose units, or both glucose and fructose units. Such a composition may characterize the disaccharide and oligosaccharide byproducts of a glucan synthesis reaction. Alternatively, a saccharide herein may contain other monosaccharides in addition to glucose and fructose, such as galactose, ribose and xylose.

A saccharide hydrolyzed in certain embodiments of the disclosed invention can be an oligosaccharide. An oligosaccharide herein can have, for example, 2, 3, 4, 5, 6, 7, 8, or 9 monosaccharide units. As would be understood in the art, an oligosaccharide herein can be referenced with respect to its degree of polymerization (DP) number, which specifies the number of monomeric units in the oligosaccharide. A DP3 oligosaccharide has 3 monomeric units, for example. Thus, the oligosaccharide can be a DP3, DP4, DP5, DP6, DP7, DP8, or DP9 oligosaccharide, for example. The DP of a saccharide in certain embodiments is 3 to 7 (i.e., DP 3-7).

An oligosaccharide herein with 3 or more monosaccharide units, for example, can comprise other linkages in addition to at least one alpha-1,3 or alpha-1,6 glucosyl-glucose linkage. For example, there may also be one or more alpha-1,5 glucosyl-fructose linkages in the oligosaccharide, which are also susceptible to hydrolysis by alpha-glucosidases as shown herein.

An oligosaccharide in certain embodiments comprises only glucose monomers linked by alpha-1,3 and/or alpha-1,6 glycosidic linkages. Thus, such oligosaccharides comprise only alpha-1,3 glucosyl-glucose and/or alpha-1,6 glucosyl-glucose linkages. Examples of such an oligosaccharide contain only alpha-1,3 linkages or alpha-1,6 linkages. An oligosaccharide can comprise at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% glucosyl-glucose linkages in certain embodiments. In other embodiments, there can be about 75-85% alpha-1,3 glucosyl-glucose linkages and about 15-25% alpha-1,6 glucosyl-glucose linkages in oligosaccharides herein. Alternatively, oligosaccharides herein can comprise any percentage (any integer value between 1% and 99%) of alpha-1,3 glucosyl-glucose linkages and any percentage (any integer value between 1% and 99%) of alpha-1,6 glucosyl-glucose linkages, just so long that the total of these percentages is not greater than 100%. Any of these oligosaccharides can be in a fraction from a glucan synthesis reaction that produces (i) an insoluble alpha-glucan (e.g., poly alpha-1,3-glucan), or (ii) a soluble alpha-glucan product, for example. This link-

age content can characterize (i) each oligosaccharide individually, or (ii) a group of oligosaccharides (i.e., average linkage content). Oligosaccharides comprising only glucose monomers linked by alpha-1,3 and/or alpha-1,6 glycosidic linkages can be DP2-DP7, or DP3-DP7, for example. It should be understood that the exact distribution of linkages in oligosaccharides can vary depending on the conditions of the glucan synthesis reaction (e.g., gtf enzyme) producing oligosaccharide byproducts. It should further be understood that the exact linkage distribution is not critical to the presently disclosed methods.

The Examples herein demonstrate that alpha-glucosidases (e.g., transglucosidase and glucoamylase enzymes) can hydrolyze both (i) leucrose, which comprises an alpha-1,5 glucosyl-fructose linkage, and (ii) oligosaccharides comprising only alpha-1,3 glucosyl-glucose and/or alpha-1,6 glucosyl-glucose linkages. Therefore, an alpha-glucosidase can be used, for example, in a reaction for hydrolyzing alpha-1,5 glucosyl-fructose linkages, alpha-1,3 glucosyl-glucose linkages and/or alpha-1,6 glucosyl-glucose linkages.

At least one alpha-1,3 or alpha-1,6 glucosyl-glucose linkage in a saccharide herein can be hydrolyzed by an alpha-glucosidase herein. Alternatively, it is believed that 2, 3, 4, 5, or more of such linkages in a saccharide can be hydrolyzed by an alpha-glucosidase, for example. Hydrolysis of at least one alpha-1,3 or alpha-1,6 glucosyl-glucose linkage can occur at the non-reducing-end of a saccharide in certain embodiments.

The amount of a saccharide is reduced in the disclosed hydrolysis method compared to the amount of the saccharide that was present prior to the contacting step. This reduction results from hydrolytic cleavage of at least one alpha-1,3 or alpha-1,6 glucosyl-glucose linkage in the saccharide. The amount (e.g., concentration) of a saccharide after the contacting step in a hydrolysis method herein can be less than about 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% (or any integer value between 1% and 90%) of the amount of the saccharide that was present prior to the contacting step (prior to contacting alpha-glucosidase with a saccharide under suitable conditions).

The amount of a saccharide is reduced in the disclosed hydrolysis method compared to the amount of the saccharide that was present prior to the contacting step. It would be understood that such a comparison can be made in any number of ways. For example, the saccharide concentration can be measured both before and after performing the hydrolysis method. Alternatively, the comparison can be made with respect to a control reaction having the same conditions, except that an alpha-glucosidase as presently disclosed is not added to the control reaction.

An alpha-glucosidase in certain embodiments herein may be immobilized. The enzyme may be immobilized using any method and/or means known in the art, such as those disclosed in U.S. Pat. Nos. 5,541,097 and 4,713,333, both of which are incorporated herein by reference. For example, one or more enzymes can be immobilized by contacting the enzyme(s) with a solution of an amine-reactive material (e.g., glutaraldehyde) to form an adduct (e.g., enzyme-glutaraldehyde adduct), after which the adduct is bonded to a solid carrier that has been treated with a polyamine (e.g., a polyethylenimine such as EPOMIN P-1050).

A solid carrier (solid support) to which an alpha-glucosidase enzyme can be immobilized in certain embodiments can be an inorganic or organic material. Such materials include, for example, gamma-alumina, titania, activated

granular carbon, granular diatomaceous earth, glass beads, porous glass, pumice-stone, silica gel, metal oxide and aluminum oxide.

A polyamine can be used to treat a solid carrier such that subsequent exposure of the solid carrier to an adduct comprising an enzyme and amine-reactive material leads to attachment of the enzyme to the solid carrier. Examples of polyamines useful herein include polyethylenediamine, a polyethylenimine (e.g., polydiethylenetriamine, polytriethylenetetramine, polytetraethylenepentamine, polyhexamethylenediamine), polymethylenedicyclohexylamine, polymethylenedianiline, polytetraethylenepentamine, polyphenylenediamine and blends of two or more of these polyamine compounds. Preferred polyamines are water-soluble and/or have a molecular weight of about from 500 to 100,000 Daltons. A polyethylenimine such as EPOMIN P-1050 can be used in certain embodiments.

An amine-reactive material useful for preparing an adduct comprising an enzyme herein can be, for example, an aldehyde, organic halide, anhydride, azo compound, isothiocyanate, and/or isocyanate. Examples of these amine-reactive materials include glutaraldehyde, succinaldehyde, terephthaldehyde, bis-diazobenzidine-2,2'-disulfonic acid, 4,4'-difluoro-3,3'-dinitrodiphenylsulfone, diphenyl-4,4'-dithiocyanate-2,2'-disulfonic acid, 3-methoxydiphenylmethane-4,4'-diisocyanate, toluene-2-isocyanate-4-isothiocyanate, toluene-2,-4-diisothiocyanate, diazobenzidine, diazobenzidine-3,3'-dianisidine, N,N'-hexamethylene bisiodoacetamide, hexamethylene diisocyanate, cyanuric chloride, and/or 1,5-difluoro-2,4-dinitrobenzene. Preferably, the amine-reactive material is an aldehyde such as glutaraldehyde.

An alpha-glucosidase enzyme adducted with an amine-reactive compound can be contacted with a polyamine-treated solid carrier, thereby immobilizing the enzyme onto the solid carrier. An immobilized enzyme herein can be employed in various reactor systems, such as in a column (e.g., packed column) or stirred tank reactor, for performing hydrolysis reaction as disclosed herein.

Suitable conditions for contacting a saccharide herein with an alpha-glucosidase (e.g., transglucosidase) are those conditions that support the hydrolysis of one or more alpha-1,3 or alpha-1,6 glucosyl-glucose linkages in the saccharide by the alpha-glucosidase. Examples of suitable conditions are disclosed in the below Examples. Conditions (e.g., temperature, pH, time) for contacting an alpha-glucosidase with a sugar substrate are also disclosed in U.S. Pat. Appl. Publ. No. 2008/0229514, U.S. Pat. No. 7,413,887 and U.S. Pat. Appl. Publ. No. 2013/0102035 (all of which are incorporated herein by reference), and may also be applicable to the disclosed hydrolysis method.

The disaccharides and oligosaccharides in the disclosed hydrolysis method are typically soluble in water or an aqueous solution. Thus, contacting a saccharide herein with an alpha-glucosidase is preferably performed under suitable conditions that are aqueous, in which the saccharide is dissolved. Aqueous conditions can characterize a solution or mixture comprising at least about 20 wt % water. Alternatively, aqueous conditions herein are at least about 20, 30, 40, 50, 60, 70, 80, 85, 90, or 95 wt % water (or any integer value between 20 and 95 wt %), for example. Aqueous conditions can further comprise a buffer, for example, such as an acidic, neutral, or alkaline buffer, at a suitable concentration and selected based on the pH range provided by the buffer. Examples of buffers/buffering agents include citrate, acetate (e.g., sodium acetate),  $\text{KH}_2\text{PO}_4$ , MOPS, CHES, borate, sodium carbonate, and sodium bicarbonate.

The pH of a hydrolysis reaction herein can be about 3.0 to 9.0, for example. Hydrolysis reaction pH can be, for example, about 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, or 9.0. Alternatively, the pH can be about 4-5. Techniques for setting pH include the use of buffers, alkalis, and/or acids, for example, and are well known in the art.

The temperature of a hydrolysis reaction herein can be about 20° C. to about 80° C., for example. Hydrolysis reaction temperature can be, for example, about 20, 30, 40, 50, 60, 70, or 80° C. (or any integer value between 20 and 80° C.). A hydrolysis temperature of about 60° C., 65° C., or 60-65° C. is preferred in certain embodiments.

A hydrolysis reaction herein can be performed for a period of at least about 10 minutes to about 90 hours, for example. The time of a hydrolysis reaction can be, for example, at least about 0.5, 1, 2, 3, 4, 8, 12, 16, 20, 24, 30, 36, 42, 48, 54, 60, 66, 72, 78, 84, or 90 hours (or any integer value between 0.5 and 72 hours). In certain embodiments, a hydrolysis reaction can be performed in less than 4 hours (e.g., 0.5-4 hours) for example. The time period required to achieve a desired level of hydrolysis will vary on the exact conditions used, and would be understood by one skilled in the art. For example, increasing the amount of enzyme added to a reaction or immobilized on a solid support used in a reaction will reduce the contact time.

One or more of alpha-glucosidase enzymes herein may be used in a hydrolysis reaction in certain embodiments. Both a transglucosidase and glucoamylase can be used in a reaction, for example. The amount of an alpha-glucosidase in a hydrolysis reaction herein can be plus/minus 10% to 20% (or 5% to 10%) from any of the amounts used in the Examples below (e.g., Example 2), for example. Alternatively, about 0.1-0.5 vol % or 0.1-1.0 vol % of alpha-glucosidase can be used in a hydrolysis reaction. Alternatively still, an alpha-glucosidase herein can be used at about, or at least about, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 ppm in a hydrolysis reaction. A transglucosidase unit (TGU) can be defined as the amount of a transglucosidase enzyme that will produce one micromole of panose per minute under the conditions of the following assay, for example. Transglucosidase activity can be assayed as follows, for example: a transglucosidase is brought up in 100 mM sodium acetate buffer, pH 4.5, containing 4 mM paranitrophenyl-alpha-glucoside and 1 mg/ml bovine serum albumin (BSA). After 30 min incubation at 30° C., the reaction is terminated by the addition of an equal volume 1 M sodium carbonate and  $\text{OD}_{405}$  is recorded. A glucoamylase unit (GAU) can be defined, for example, as the amount of glucoamylase enzyme that will produce 1 g of reducing sugar calculated as glucose per hour from a soluble starch substrate (4% DS [degree of substitution]) at pH 4.2 and 60° C.

The initial concentration of a saccharide in a hydrolysis reaction in certain embodiments of the disclosed invention can be about 1 wt % to 50 wt %, for example. For example, the concentration of leucrose can be about 5, 10, 15, 20, 25, 30, 35, or 40 wt % (or any integer value between 5 and 40 wt %). As another example, the concentration of one or more oligosaccharides (e.g., DP2, DP3, DP4, DP2-DP7, DP3-DP7) in a hydrolysis reaction herein can be about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 wt %. Those skilled in the art would recognize that the concentration of total sugars (which includes disaccharides and oligosaccharides) can have an impact on the activity of alpha-glucosidase enzymes; preferred concentrations of total sugars in a hydrolysis reaction to maximize enzyme activity can be less than

50 wt % dry solids (DS), with a most preferred concentration of 20-35 wt % DS in some aspects.

Suitable conditions in certain embodiments for contacting a saccharide with an alpha-glucosidase herein can comprise (i) a glucan synthesis reaction, or (ii) a fraction obtained from a glucan synthesis reaction, where the saccharide is a byproduct of the glucan synthesis reaction. In other words, a hydrolysis reaction herein may be conducted in the context of a glucan synthesis reaction or a fraction of a glucan synthesis reaction, though it is typically conducted in the latter. A glucan synthesis reaction herein can produce one or more insoluble and/or soluble alpha-glucan products, for example. Thus, a glucan synthesis reaction can be characterized in some embodiments herein as an "alpha-glucan synthesis reaction".

A glucan synthesis reaction generally refers to a solution comprising at least sucrose, water and one active glucosyltransferase enzyme, and optionally other components. Other components that can be in a glucan synthesis reaction include fructose, glucose, leucrose, soluble oligosaccharides (e.g., DP2-DP7), and soluble glucan product(s). Also, one or more alpha-glucanohydrolase enzymes can be comprised in a glucan synthesis reaction in some aspects. It would be understood that certain glucan products, such as poly alpha-1,3-glucan with a DP of at least 8 or 9, may be water-insoluble and thus are not dissolved in a glucan synthesis reaction, but rather may be present out of solution. Thus, a glucan produced by glucan synthesis reaction herein can be insoluble. An alpha-glucosidase enzyme herein can be added to a glucan synthesis reaction at any stage thereof, such as during initial preparation of the reaction or when the reaction is near (e.g., 80 to 90% complete) or at completion, where the latter two time points are preferred.

A glucan synthesis reaction herein may be one that, in addition to producing a glucan product, produces byproducts such as leucrose and/or soluble oligosaccharides. A glucan in some aspects is a poly alpha-glucan. Thus, a glucan synthesis reaction herein can be for producing poly alpha-1,3-glucan or mutan, for example, which are typically co-produced with at least leucrose and/or oligosaccharide byproducts in a glucan synthesis reaction.

A glucan synthesis reaction in certain embodiments comprises a glucosyltransferase enzyme that produces a poly alpha-glucan such as poly alpha-1,3-glucan. Examples of such glucosyltransferase enzymes useful herein are disclosed in U.S. Pat. No. 7,000,000, and U.S. Pat. Appl. Publ. Nos. 2013/0244288, 2013/0244287 and 2014/0087431 (all of which are incorporated herein by reference).

A glucosyltransferase enzyme herein may be derived from any microbial source, such as a bacteria or fungus. Examples of bacterial glucosyltransferase enzymes are those derived from a *Streptococcus* species, *Leuconostoc* species or *Lactobacillus* species. Examples of *Streptococcus* species include *S. salivarius*, *S. sobrinus*, *S. dentirosetti*, *S. downei*, *S. mutans*, *S. oralis*, *S. galloyticus* and *S. sanguinis*. Examples of *Leuconostoc* species include *L. mesenteroides*, *L. arabinosum*, *L. argentinum*, *L. camosum*, *L. citreum*, *L. cremoris*, *L. dextranicum* and *L. fructosum*. Examples of *Lactobacillus* species include *L. acidophilus*, *L. delbrueckii*, *L. helveticus*, *L. salivarius*, *L. casei*, *L. curvatus*, *L. plantarum*, *L. sakei*, *L. brevis*, *L. buchneri*, *L. fermentum* and *L. reuteri*.

A glucosyltransferase enzyme herein can be primer-independent or primer-dependent. Primer-independent glucosyltransferase enzymes do not require the presence of a primer to perform glucan synthesis. A primer-dependent glucosyltransferase enzyme requires the presence of an initiating

molecule in the reaction solution to act as a primer for the enzyme during glucan polymer synthesis. The term "primer" as used herein refers to any molecule that can act as the initiator for a glucosyltransferase enzyme. Primers that can be used in certain embodiments include dextran and other carbohydrate-based primers, such as hydrolyzed glucan, for example. U.S. Appl. Publ. No. 2013/0244287, which is incorporated herein by reference, discloses preparation of hydrolyzed glucan using poly alpha-1,3-glucan as the starting material. Dextran for use as a primer can be dextran T10 (i.e., dextran having a molecular weight of 10 kD), for example.

A glucosyltransferase enzyme for a glucan synthesis reaction herein may be produced by any means known in the art. For example, a glucosyltransferase enzyme may be produced recombinantly in a heterologous expression system, such as a microbial heterologous expression system. Examples of heterologous expression systems include bacterial (e.g., *E. coli* such as TOP10 or MG1655; *Bacillus* sp.) and eukaryotic (e.g., yeasts such as *Pichia* sp. and *Saccharomyces* sp.) expression systems.

A glucosyltransferase enzyme described herein may be used in any purification state (e.g., pure or non-pure). For example, a glucosyltransferase enzyme may be purified and/or isolated prior to its use. Examples of glucosyltransferase enzymes that are non-pure include those in the form of a cell lysate. A cell lysate or extract may be prepared from a bacteria (e.g., *E. coli*) used to heterologously express the enzyme. For example, the bacteria may be subjected to disruption using a French pressure cell. In alternative embodiments, bacteria may be homogenized with a homogenizer (e.g., APV, Rannie, Gaulin). A glucosyltransferase enzyme is typically soluble in these types of preparations. A bacterial cell lysate, extract, or homogenate herein may be used at about 0.15-0.3% (v/v), for example, in a reaction solution for producing a poly alpha-glucan such as poly alpha-1,3-glucan from sucrose.

The temperature of a glucan synthesis reaction herein can be controlled, if desired. In certain embodiments, the temperature of the reaction is between about 5° C. to about 50° C. The temperature in certain other embodiments is between about 20° C. to about 40° C.

The initial concentration of sucrose in a glucan synthesis reaction herein can be about 20 g/L to about 400 g/L, for example. Alternatively, the initial concentration of sucrose can be about 75 g/L to about 175 g/L, or from about 50 g/L to about 150 g/L. Alternatively still, the initial concentration of sucrose can be about 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, or 160 g/L (or any integer value between 40 and 160 g/L), for example. "Initial concentration of sucrose" refers to the sucrose concentration in a gtf reaction solution just after all the reaction solution components have been added (at least water, sucrose, gtf enzyme).

Sucrose used in a glucan synthesis reaction herein can be highly pure (≥99.5%) or be of any other purity or grade. For example, sucrose can have a purity of at least 99.0%, or can be reagent grade sucrose. As another example, incompletely refined sucrose can be used. Incompletely refined sucrose herein refers to sucrose that has not been processed to white refined sucrose. Thus, incompletely refined sucrose can be completely unrefined or partially refined. Examples of unrefined sucrose are "raw sucrose" ("raw sugar") and solutions thereof. Examples of partially refined sucrose have not gone through one, two, three, or more crystallization steps. The ICUMSA (International Commission for Uniform Methods of Sugar Analysis) of incompletely refined sucrose herein can be greater than 150, for example. Sucrose herein may be

derived from any renewable sugar source such as sugar cane, sugar beets, cassava, sweet sorghum, or corn. Suitable forms of sucrose useful herein are crystalline form or non-crystalline form (e.g., syrup, cane juice, beet juice), for example. Additional suitable forms of incompletely refined sucrose are disclosed in U.S. Appl. No. 61/969,958.

Methods of determining ICUMSA values for sucrose are well known in the art and disclosed by the International Commission for Uniform Methods of Sugar Analysis in *ICUMSA Methods of Sugar Analysis: Official and Tentative Methods Recommended by the International Commission for Uniform Methods of Sugar Analysis (ICUMSA)* (Ed. H. C. S. de Whalley, Elsevier Pub. Co., 1964), for example, which is incorporated herein by reference. ICUMSA can be measured, for example, by ICUMSA Method GS1/3-7 as described by R. J. McCowage, R. M. Urquhart and M. L. Burge (*Determination of the Solution Colour of Raw Sugars, Brown Sugars and Coloured Syrups at pH 7.0-Official*, Verlag Dr Albert Bartens, 2011 revision), which is incorporated herein by reference.

The pH of a glucan synthesis reaction in certain embodiments can be between about 4.0 to about 8.0. Alternatively, the pH can be about 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, or 8.0. The pH can be adjusted or controlled by the addition or incorporation of a suitable buffer, including but not limited to: phosphate, tris, citrate, or a combination thereof. Buffer concentration in a glucan synthesis reaction can be from 0 mM to about 100 mM, or about 10, 20, or 50 mM, for example.

Poly alpha-1,3-glucan produced in a glucan synthesis reaction herein may have at least about 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% (or any integer value between 50% and 100%) glycosidic linkages that are alpha-1,3. In such embodiments, accordingly, the poly alpha-1,3-glucan has less than about 50%, 40%, 30%, 20%, 10%, 5%, 4%, 3%, 2%, 1%, or 0% (or any integer value between 0% and 50%) of glycosidic linkages that are not alpha-1,3.

Poly alpha-1,3-glucan herein preferably has a backbone that is linear/unbranched. In certain embodiments, the poly alpha-1,3-glucan has no branch points or less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% branch points as a percent of the glycosidic linkages in the polymer. Examples of branch points include alpha-1,6 branch points.

The molecular weight of poly alpha-1,3-glucan produced in a glucan synthesis reaction herein can be measured as number-average molecular weight ( $M_n$ ) or weight-average molecular weight ( $M_w$ ). Alternatively, molecular weight can be measured in Daltons or grams/mole. It may also be useful to refer to the  $DP_w$  (weight average degree of polymerization) or  $DP_n$  (number average degree of polymerization) of the poly alpha-1,3-glucan polymer.

The  $M_n$  or  $M_w$  of poly alpha-1,3-glucan herein may be at least about 1000. Alternatively, the  $M_n$  or  $M_w$  can be at least about 1000 to about 600000 (or any integer value between 1000 and 600000), for example. Alternatively still, poly alpha-1,3-glucan can have a molecular weight in  $DP_n$  or  $DP_w$  of at least about 100, or of at least about 100 to 1000 (or any integer value between 100 and 1000).

A fraction of a glucan synthesis reaction may constitute suitable conditions for contacting a saccharide with an alpha-glucosidase as presently disclosed. A fraction can be a portion of, or all of, the liquid solution from a glucan synthesis reaction. Typically, a fraction has been separated from soluble or insoluble glucan product(s) synthesized in the reaction. For example, a fraction can be separated from one or more glucan products that are insoluble in water (e.g.,

poly alpha-1,3-glucan) which fall out of solution during their synthesis. A fraction in certain preferred embodiments of the present disclosure is from a poly alpha-1,3-glucan synthesis reaction.

The volume of a fraction (before optionally diluting or concentrating the fraction, see below) in certain embodiments can be at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% (or any integer value between 10% and 90%) of the volume of the glucan synthesis reaction from which it is obtained. Typically, in glucan synthesis reactions producing an insoluble glucan (e.g., poly alpha-1,3-glucan), the fraction will be a portion of (not all of) the liquid solution component of the reaction. A fraction can be obtained at any stage of a glucan synthesis reaction, but is preferably obtained near (e.g., greater than 80 or 90% complete) or after completion of the reaction.

Examples of a fraction of a glucan synthesis reaction in certain embodiments include filtrates and supernatants. Thus, in those embodiments in which an insoluble glucan product is synthesized, a fraction herein can be obtained (separated) from a glucan synthesis reaction using a funnel, filter (e.g., press filter), centrifuge, or any other method or equipment known in the art that allows removal of some or all liquids from solids. Filtration can be by gravity, vacuum, or press filtration, for example. Filtration preferably removes all or most of an insoluble glucan; any filter material (e.g., filter paper) with an average pore size (e.g., ~40-50 micron) sufficient to remove solids from liquids can be used. A fraction typically retains all or most of its dissolved components, such as byproducts of the glucan synthesis reaction.

A fraction herein can optionally be diluted or concentrated, if desired. Concentration of a fraction can be performed using any other method or equipment known in the art suitable for concentrating a solution. For example, a fraction can be concentrated by evaporation, such as with a rotary evaporator (e.g., set at a temperature of about 40-50° C.). A fraction in some aspects herein can be concentrated down to a volume that is about 75%, 80%, 85%, 90%, or 95% of the original fraction volume. A concentrated fraction (e.g., concentrated filtrate) can optionally be referred to as a syrup.

A fraction in some aspects can comprise water that replaces the water that was present in the composition from which the fraction was obtained. For example, saccharide byproduct(s) from a glucan synthesis reaction can be separated in certain chromatographic methods in which the original solvent is replaced with another solvent (e.g., saccharide byproducts that are bound to a column [thus removed from the original solvent] can be eluted into a new solvent).

A fraction in some aspects may be treated in a manner to have any of the suitable conditions (e.g., temperature, pH and time) disclosed above for contacting a saccharide with an alpha-glucosidase. For example, a fraction can be modified to have a pH of about 4 to 5 before an alpha-glucosidase is added to the fraction. As another example, the temperature of a hydrolysis reaction with a fraction can be about 55-65° C. (e.g., about 60° C.). A fraction that has been concentrated down to a syrup can be used in a hydrolysis reaction in yet another example.

A fraction in certain preferred embodiments herein is from a poly alpha-1,3-glucan synthesis reaction; such a fraction is preferably a filtrate. A fraction of a poly alpha-1,3-glucan synthesis reaction herein comprises at least water, fructose and one or more types of saccharide (leucrose and/or oligosaccharides such as DP2-DP7). Other components that may be in this type of fraction include

sucrose (i.e., residual sucrose not consumed in the gtf reaction), one or more gtf enzymes, glucose, buffer, salts, FermaSure®, borates, sodium hydroxide, hydrochloric acid, cell lysate components, proteins and/or nucleic acids, for example. Minimally, the components of a fraction from a poly alpha-1,3-glucan synthesis reaction include water, fructose, glucose, one or more types of saccharide (leucrose and/or oligosaccharides such as DP2-DP7), and optionally sucrose, for example. It would be understood that the composition of a fraction depends, in part, on the conditions of the glucan synthesis reaction from which the fraction is obtained. In those fractions containing one or more gtf enzymes, it is preferable that such one or more gtf enzymes are deactivated (e.g., heat-deactivated) before using the fraction in a hydrolysis reaction herein.

It should be understood that the exact distribution of sugar byproducts produced via polymerization of sucrose in a glucan synthesis reaction can vary based on the reaction conditions and gtf enzyme used, especially on temperature and sucrose concentration. It should also be understood that the exact composition of sugars in a fraction of a glucan synthesis reaction is not critical to the disclosed hydrolysis process. Generally, as the amount of sucrose is increased, the selectivity of the reaction towards both leucrose and oligosaccharides will increase. Conversely, as the temperature increases, the selectivity of the reaction towards leucrose tends to decrease, while the selectivity towards oligosaccharides is largely unaffected. It should also be understood that the ratio of sugars to water, i.e., wt % dry solids (DS), which is calculated by dividing the mass of sugar to total solution weight, can be adjusted either by evaporating water, preferably at temperatures below 50° C. under vacuum, or addition of water, without significant impact to the relative distribution of sugars in a fraction of a glucan synthesis reaction. It is also possible to increase the percentage of sucrose in a fraction by stopping the gtf reaction before complete conversion (to glucan) is achieved, either by reducing the pH below the active range for the gtf enzyme or by thermal deactivation of the gtf enzyme.

In certain embodiments, a glucan synthesis reaction herein can produce one or more soluble alpha-glucan products. A soluble alpha-glucan product ("soluble fiber", alternatively) can be (i) a direct product of a glucosyltransferase, or (ii) a product of the concerted action of both a glucosyltransferase and an alpha-glucanohydrolase capable of hydrolyzing glucan polymers having one or more alpha-1,3-glycosidic linkages or one or more alpha-1,6-glycosidic linkages.

A soluble alpha-glucan herein can comprise, for example:

- a) at least 75% alpha-1,3-glycosidic linkages;
- b) less than 25% alpha-1,6-glycosidic linkages;
- c) less than 10% alpha-1,3,6-glycosidic linkages;
- d) an  $M_w$  of less than 5000 Daltons;
- e) a viscosity of less than 0.25 Pascal second (Pa·s) at 12 wt % in water at 20° C.;
- f) a dextrose equivalence (DE) in the range of 4 to 40;
- g) a digestibility of less than 10% as measured by the Association of Analytical Communities (AOAC) method 2009.01;
- h) a solubility of at least 20% (w/w) in pH 7 water at 25° C.; and
- i) a polydispersity index (PDI) of less than 5.

Such a soluble alpha-glucan can be produced as disclosed in U.S. Appl. No. 62/004,290.

As an example, a soluble alpha-glucan fiber composition can comprise at least 75%, preferably at least 80%, more

preferably at least 85%, even more preferably at least 90%, and most preferably at least 95% alpha-(1,3) glycosidic linkages.

As another example, in addition to the alpha-(1,3) glycosidic linkage embodiments described above, a soluble alpha-glucan fiber composition can further comprise less than 25%, preferably less than 10%, more preferably 5% or less, and even more preferably less than 1% alpha-(1,6) glycosidic linkages.

As another example, in addition to the alpha-(1,3) and alpha-(1,6) glycosidic linkage content embodiments described above, a soluble alpha-glucan fiber composition can further comprise less than 10%, preferably less than 5%, and most preferably less than 2.5% alpha-(1,3,6) glycosidic linkages.

As another example, a soluble alpha-glucan fiber composition can comprise 93 to 97% alpha-(1,3) glycosidic linkages and less than 3% alpha-(1,6) glycosidic linkages and has a weight-average molecular weight corresponding to a DP of 3 to 7 mixture. In a further embodiment, a soluble alpha-glucan fiber composition can comprise about 95% alpha-(1,3) glycosidic linkages and about 1% alpha-(1,6) glycosidic linkages and has a weight-average molecular weight corresponding to a DP of 3 to 7 mixture. In a further aspect of the above embodiment, a soluble alpha-glucan fiber composition can further comprise 1 to 3% alpha-(1,3,6) linkages or preferably about 2% alpha-(1,3,6) linkages.

As another example, in addition to the above-mentioned glycosidic linkage content embodiments, a soluble alpha-glucan fiber composition can further comprise less than 5%, preferably less than 1%, and most preferably less than 0.5% alpha-(1,4) glycosidic linkages.

As another example, in addition to the above-mentioned glycosidic linkage content embodiments, a soluble alpha-glucan fiber composition can comprise a weight average molecular weight ( $M_w$ ) of less than 5000 Daltons, preferably less than 2500 Daltons, more preferably between 500 and 2500 Daltons, and most preferably about 500 to about 2000 Daltons.

As another example, in addition to any of the above features, a soluble alpha-glucan fiber composition can comprise a viscosity of less than 250 centipoise (0.25 Pa·s), preferably less than 10 cP (0.01 Pa·s), preferably less than 7 cP (0.007 Pa·s), more preferably less than 5 cP (0.005 Pa·s), more preferably less than 4 cP (0.004 Pa·s), and most preferably less than 3 cP (0.003 Pa·s) at 12 wt % in water at 20° C.

A soluble alpha-glucan fiber composition can have, in certain embodiments, a digestibility of less than 10%, or preferably less than 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% digestibility as measured by the Association of Analytical Communities (AOAC) method 2009.01. In another aspect, the relative level of digestibility may alternatively be determined using AOAC 2011.25 (Integrated Total Dietary Fiber Assay) (McCleary et al., 2012, *J. AOAC Int.*, 95 (3), 824-844).

In addition to any of the above embodiments, a soluble alpha-glucan fiber composition can have a solubility of at least 20% (w/w), preferably at least 30%, 40%, 50%, 60%, or 70% in pH 7 water at 25° C.

In one embodiment, a soluble alpha-glucan fiber composition can comprise a reducing sugar content of less than 10 wt %, preferably less than 5 wt %, and most preferably 1 wt % or less.

In one embodiment, a soluble alpha-glucan fiber composition can comprise a caloric content of less than 4 kcal/g,

## 25

preferably less than 3 kcal/g, more preferably less than 2.5 kcal/g, and most preferably about 2 kcal/g or less.

As another example, a soluble alpha-glucan herein can comprise:

a) 10% to 30% alpha-1,3-glycosidic linkages;  
 b) 65% to 87% alpha-1,6-glycosidic linkages;  
 c) less than 5% alpha-1,3,6-glycosidic linkages;  
 d) a weight average molecular weight (Mw) of less than 5000 Daltons;

e) a viscosity of less than 0.25 Pascal second (Pa·s) at 12 wt % in water at 20° C.;

f) a dextrose equivalence (DE) in the range of 4 to 40, preferably 10 to 40;

g) a digestibility of less than 10% as measured by the Association of Analytical Communities (AOAC) method 2009.01;

h) a solubility of at least 20% (w/w) in pH 7 water at 25° C.; and

i) a polydispersity index (PDI) of less than 5.

Such a soluble alpha-glucan can be produced as disclosed in U.S. Appl. No. 62/004,308.

As another example, a soluble alpha-glucan herein can comprise:

a) 25-35 alpha-1,3-glycosidic linkages;  
 b) 55-75% alpha-1,6-glycosidic linkages;  
 c) 5-15% alpha-1,3,6-glycosidic linkages;  
 d) a weight average molecular weight of less than 5000 Daltons;

e) a viscosity of less than 0.25 Pascal second (Pa·s) at 12 wt % in water at 20° C.;

f) a dextrose equivalence (DE) in the range of 4 to 40;

g) a digestibility of less than 10% as measured by the Association of Analytical Communities (AOAC) method 2009.01;

h) a solubility of at least 20% (w/w) in water at 25° C.; and

i) a polydispersity index of less than 5.

Such a soluble alpha-glucan can be produced as disclosed in U.S. Appl. No. 62/004,312.

As another example, a soluble alpha-glucan herein can comprise:

a) at least 95% alpha-1,6-glycosidic linkages;  
 b) 1% or less alpha-1,3-glycosidic linkages;  
 c) less than 2% alpha-1,3,6-glycosidic linkages;  
 d) less than 1.5% alpha-1,4-glycosidic linkages;  
 e) a weight average molecular weight of less than 20000 Daltons;

f) a viscosity of less than 0.25 Pascal second (Pa·s) at 12 wt % in water at 20° C.;

g) a dextrose equivalence (DE) in the range of 1 to 30;

h) a digestibility of less than 10% as measured by the Association of Analytical Communities (AOAC) method 2009.01;

i) a solubility of at least 20% (w/w) in pH 7 water at 25° C.; and

j) a polydispersity index of less than 5.

Such a soluble alpha-glucan can be produced as disclosed in U.S. Appl. No. 62/004,314.

As another example, a soluble alpha-glucan herein can comprise:

a) a range of:

i) 1% to 50% of alpha-1,3-glycosidic linkages; or

ii) greater than 10% but less than 40% alpha-1,4-glycosidic linkages; or

iii) any combination of i) and ii);

b) 1 to 50% alpha-1,2-glycosidic linkages;

c) 0-25% alpha-1,3,6-glycosidic linkages;

## 26

d) less than 98% alpha-1,6-glycosidic linkages;

e) a weight average molecular weight of less than 300 kDa;

f) a viscosity of less than 0.25 Pascal second (Pa·s) at 12 wt % in water at 20° C.;

g) a digestibility of less than 20% as measured by the Association of Analytical Communities (AOAC) method 2009.01;

h) a solubility of at least 20% (w/w) in pH 7 water at 25° C.; and

i) a polydispersity index of less than 26, preferably less than 5.

Such a soluble alpha-glucan can be produced as disclosed in U.S. Appl. No. 62/004,305.

In certain embodiments, a soluble alpha-glucan is a direct product of a glucosyltransferase. Such a glucosyltransferase, and conditions for use thereof in a suitable glucan synthesis reaction, can be as disclosed herein, or as disclosed in any of U.S. Patent Appl. Nos. 62/004,290, 62/004,308, 62/004,312, 62/004,314, and/or 62/004,305, for example.

A soluble alpha-glucan can alternatively be a product, for example, of the concerted action of both a glucosyltransferase and an alpha-glucanohydrolase that is capable of hydrolyzing glucan polymers having one or more alpha-1,3-glycosidic linkages or one or more alpha-1,6-glycosidic linkages. In some aspects, a glucan synthesis reaction for producing a soluble alpha-glucan product can comprise both at least one glucosyltransferase and at least one alpha-glucanohydrolase. In other aspects, a glucan synthesis reaction can initially comprise one or more glucosyltransferases as the only enzyme component(s). Such a reaction produces a first alpha-glucan product that has not yet been subject to modification by an alpha-glucanohydrolase. Then, at least one alpha-glucanohydrolase is added to the reaction for a suitable period of time to allow modification of the first product to a soluble alpha-glucan product. Thus, there are different ways by which to synthesize a soluble alpha-glucan product through the concerted action of both a glucosyltransferase and an alpha-glucanohydrolase. Conditions for performing a glucan synthesis reaction in which one or more alpha-glucanohydrolase enzymes are included during glucan synthesis reaction and/or after glucan synthesis can be as disclosed herein, or as disclosed in any of U.S. Patent Appl. Nos. 62/004,290, 62/004,308, 62/004,312, 62/004,314, and/or 62/004,305, for example.

An alpha-glucanohydrolase herein can be, for example, a dextranase (capable of hydrolyzing alpha-1,6-linked glycosidic bonds; E.C. 3.2.1.11), a mutanase (capable of hydrolyzing alpha-1,3-linked glycosidic bonds; E.C. 3.2.1.59), a mycodextranase (capable of endohydrolysis of (1-4)-alpha-D-glycosidic linkages in alpha-D-glucans containing both (1-3)- and (1-4)-bonds; EC 3.2.1.61), a glucan 1,6-alpha-glucosidase (EC 3.2.1.70), and an alternanase (capable of endohydrolytically cleaving alternan; E.C. 3.2.1.-; see U.S. Pat. No. 5,786,196).

A mutanase comprising SEQ ID NO:47 can be used in certain aspects. Alternatively, a mutanase can comprise an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:47 and have mutanase activity, for example.

A glucan synthesis reaction as presently disclosed for producing one or more soluble alpha-glucan products can serve directly as suitable conditions in which to perform a hydrolysis reaction herein in which an alpha-glucosidase is used to hydrolyze an alpha-1,5 glucosyl-fructose linkage. Such hydrolysis can be performed following any of the conditions disclosed above regarding hydrolytic treatment

of a glucan synthesis reaction that produces poly alpha-1, 3-glucan, for example. Alternatively, a fraction (e.g., chromatographic fraction) of a glucan synthesis reaction for producing one or more soluble alpha-glucan products can be used as suitable conditions in which to perform alpha-glucosidase-mediated hydrolysis of alpha-1,5 glucosyl-fructose linkages.

A fraction in certain embodiments herein can be a chromatographic fraction of a glucan synthesis reaction. For example, a fraction can be a chromatographic fraction of a glucan synthesis reaction that produces one or more soluble alpha-glucan products as disclosed herein. Such a reaction can optionally include one or more alpha-glucanohydrolases during glucan synthesis, and/or after completion of glucan synthesis. A fraction in any of these types of embodiments typically has been obtained for the purpose of separating all of, or most of (e.g., at least about 60%, 70%, 80%, 90%, 95%), a soluble alpha-glucan product from a reaction composition from which it was produced. Once separated from all or most of a soluble alpha-glucan product, a fraction can be subjected to any of the alpha-1,5 glucosyl-fructose hydrolysis processes disclosed herein using one or more alpha-glucanases.

A chromatographic fraction herein can typically be obtained using a suitable type of liquid chromatography. Liquid chromatography can be performed using size-exclusion chromatography (SEC), column chromatography, high-performance liquid chromatography (HPLC), ion-exchange chromatography, affinity chromatography, ultrafiltration, microfiltration, or dialysis, for example.

The disclosed invention also concerns a composition produced by contacting a saccharide with an alpha-glucosidase (e.g., transglucosidase), wherein (i) the saccharide is a disaccharide or oligosaccharide comprising at least one alpha-1,3 or alpha-1,6 glucosyl-glucose linkage, and (ii) the alpha-glucosidase hydrolyzes at least one alpha-1,3 or alpha-1,6 glucosyl-glucose linkage of the saccharide. The composition produced in this manner comprises a reduced amount of the saccharide compared to the amount of the saccharide that was present prior to the contacting. Examples of the composition include any of those disclosed herein, such as a hydrolyzed filtrate from a glucan synthesis reaction, or a hydrolyzed fraction of a glucan synthesis reaction used to produce soluble alpha-glucan. Any of the features disclosed above and in the Examples regarding a hydrolysis method and products thereof can characterize the composition. The following features of the composition are examples.

An alpha-glucosidase enzyme in certain embodiments of the composition can comprise an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:5, 6, 8, 9, 11, 12, 14, 15, 17, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, or that of DIAZYME RDF ULTRA (DuPont Industrial Biosciences). A transglucosidase in certain embodiments of the composition can comprise an amino acid sequence that is at least 90% identical to SEQ ID NO:1. Alternatively, any of the alpha-glucosidases disclosed herein can be used to produce the disclosed composition.

A saccharide in certain embodiments of the composition has a degree of polymerization before hydrolysis of 3 to 7.

A composition produced by a hydrolysis method herein can have, for example, a concentration of a saccharide that is less than 50% of the concentration of the saccharide that was present prior to contacting the saccharide with an alpha-glucosidase.

A composition produced by a hydrolysis method in certain embodiments herein can be a glucan synthesis reaction, or a fraction thereof, in which a saccharide byproduct of the glucan synthesis reaction is contacted with an alpha-glucosidase. A fraction in this embodiment can be a filtrate of the glucan synthesis reaction, or a fraction of a glucan synthesis reaction used to produce soluble alpha-glucan, for example. The saccharide in this embodiment can have a degree of polymerization of 3 to 7 before hydrolysis, for example.

It would be understood by a skilled artisan that the presently disclosed embodiments are useful, in part, for saccharifying disaccharides and oligosaccharides that can otherwise be difficult to breakdown. This feature can be taken advantage of to perform enhanced methods of (i) fructose enrichment and (ii) fermentation, for example.

Example 6 below demonstrates that fructose enrichment by chromatography is enhanced when using a glucan filtrate hydrolyzed by an alpha-glucosidase (transglucosidase), as compared to using a filtrate that was not hydrolyzed.

Thus, the disclosed invention further concerns a method of enriching fructose that is present in a fraction of a glucan synthesis reaction. This method comprises (a) contacting a fraction obtained from a glucan synthesis reaction with an alpha-glucosidase (e.g., transglucosidase) under suitable conditions, wherein the enzyme hydrolyzes at least one alpha-1,3 or alpha-1,6 glucosyl-glucose linkage of a disaccharide or oligosaccharide comprised within the fraction; and (b) separating fructose from the hydrolyzed fraction of step (a) to obtain a composition having a higher concentration of fructose compared to the fructose concentration of the fraction of step (a).

The features of the disclosed fructose enrichment method regarding alpha-glucosidase (e.g., transglucosidase) enzymes, and fractions of a glucan synthesis reaction, for example, can be according to any of the disclosures provided herein concerning each of these features.

Step (b) of separating fructose can be performed by any means known in the art. For example, chromatography can be employed as disclosed in the below Examples, or by following the disclosure of European Patent Publ. No. EP2292803B1, which is incorporated herein by reference.

A composition (e.g., fructose solution or fructose syrup) having a higher concentration of fructose resulting from the disclosed enrichment method can have at least about 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 wt % fructose.

A fructose enrichment method herein can perform better than one which utilizes a filtrate that has not been hydrolyzed with an alpha-glucosidase as presently disclosed. Such increased performance can be measured in terms of a percent fructose recovery of at least 40%, 45%, or 50%.

The present disclosure further concerns a fermentation method comprising (a) contacting a fraction obtained from a glucan synthesis reaction with an alpha-glucosidase enzyme (e.g., transglucosidase or glucoamylase) under suitable conditions, wherein the alpha-glucosidase enzyme hydrolyzes at least one alpha-1,5 glucosyl-fructose linkage of a disaccharide or oligosaccharide comprised within the fraction; (b) fermenting the fraction of step (a) with a microbe to yield a product; and (c) optionally, isolating the product of (b). The fermenting step of (b) can be performed after step (a) or simultaneously with step (a). Significantly, this method can be used to produce ethanol, for example, by fermenting a hydrolyzed filtrate of a glucan synthesis reaction. The ethanol yield from such a process is higher than the ethanol yield obtained when fermenting a glucan filtrate that has not been hydrolyzed.

The features of the disclosed fermentation method regarding alpha-glucosidase (e.g., transglucosidase or glucoamylase) enzymes, disaccharides and oligosaccharides, fractions of a glucan synthesis reaction, and suitable contacting conditions, for example, can be according to any of the disclosures provided herein concerning each of these features.

A microbe for use in a fermentation method herein can be a bacteria, yeast, or fungus, for example. Examples of bacteria useful herein include *Lactobacillus* species, *Streptococcus* species, *Bifidobacterium* species, *Leuconostoc* species, *Escherichia* species (e.g., *E. coli*) and *Bacillus* species. Examples of yeast useful herein include *Saccharomyces* species such as *S. cerevisiae* and *S. bayanus*.

A fermentation method herein can yield a product such as ethanol or an acid (e.g., lactic acid). It is believed, however, that other products can be produced if desired. It would be understood by one of skill in the art that production of certain products using a fermentation method as disclosed would depend on various conditions such as the microbe(s) used in the fermentation. Conditions for fermentation herein can be as disclosed in the below Examples, or as disclosed in El-Mansi et al. (2006, *Fermentation Microbiology and Biotechnology*, Second Edition, CRC Press) and Stanbury et al. (1999, *Principles of Fermentation Technology*, Second Edition, Butterworth-Heinemann), for example, which are both incorporated herein by reference.

The yield of a product in certain embodiments of a fermentation method herein is higher than the product yield obtained when fermenting a glucan filtrate that has not been hydrolyzed with an alpha-glucosidase herein. This comparison can be with respect to a control fermentation, for example, which used a non-hydrolyzed fraction of a glucan synthesis reaction. Product yield of a fermentation herein can be increased by at least about 10%, 20%, 40%, 60%, 80%, or 100% (or any integer value between 10% and 100%), for example. In addition, the rate of product formation by a fermentation herein can be increased.

Example 7 below demonstrates that leucrose can be fermented to ethanol by yeast provided a feed comprising glucan filtrate that had not been hydrolyzed. Thus, further disclosed herein is a method of using a microbe to ferment leucrose to a product (e.g., ethanol). Such a method can comprise fermenting a glucan filtrate that (i) has, or (ii) has not been, hydrolyzed with an alpha-glucosidase as disclosed herein. Regardless of whether the leucrose is provided in a glucan filtrate or another form (e.g., semi-purified or enriched form), a method for fermenting leucrose can comprise adapting a microbe (e.g., yeast such as *S. cerevisiae*) for utilizing leucrose. Such adaptation can comprise growing a microbe in the presence of leucrose, and optionally other sugars, over at least 2 or 3 growth cycles, for example, after which the microbe utilizes more leucrose for fermenting a product. In certain embodiments, a microbe can be (i) grown in a first feed comprising leucrose (1 cycle complete), (ii) removed from the first feed, (iii) grown in a second feed comprising leucrose (two cycles complete), (iv) optionally removed from the second feed, and (v) optionally grown in a third feed (three cycles complete). A microbe adapted in this manner can have an increased capacity to ferment leucrose in certain embodiments.

Example 9 below demonstrates that almost all (e.g., >98% or >99%) the leucrose present in a glucan filtrate can be used for fermentation by yeast when the glucan filtrate is hydrolyzed with a transglucosidase while at the same time fermented with yeast. Thus, an enhanced leucrose fermentation method herein can comprise hydrolysis of leucrose with an

alpha-glucosidase (e.g., transglucosidase or glucoamylase) while simultaneously fermenting the leucrose with a microbe.

Non-limiting examples of compositions and methods disclosed herein include:

1. A method of hydrolyzing an alpha-1,3 or alpha-1,6 glucosyl-glucose linkage in a saccharide comprising at least one alpha-1,3 or alpha-1,6 glucosyl-glucose linkage, wherein the saccharide is a disaccharide or oligosaccharide, and wherein the method comprises:
  - contacting the saccharide with an alpha-glucosidase enzyme under suitable conditions, wherein the alpha-glucosidase enzyme hydrolyzes at least one alpha-1,3 or alpha-1,6 glucosyl-glucose linkage of the saccharide,
  - and wherein the amount of the saccharide is reduced compared to the amount of the saccharide that was present prior to the contacting.
2. The method of embodiment 1, wherein the alpha-glucosidase enzyme is immobilized.
3. The method of embodiment 1 or 2, wherein the degree of polymerization of the saccharide before hydrolysis is 3 to 7.
4. The method of embodiment 1, 2, or 3, wherein the concentration of the saccharide after the contacting step is less than 50% of the concentration of the saccharide that was present prior to the contacting.
5. The method of embodiment 1, 2, 3, or 4, wherein the suitable conditions comprise (i) a glucan synthesis reaction, or (ii) a fraction obtained from the glucan synthesis reaction; wherein the saccharide is a byproduct of the glucan synthesis reaction.
6. The method of embodiment 5, wherein the glucan synthesis reaction produces at least one insoluble alpha-glucan product.
7. The method of embodiment 6, wherein the fraction is a filtrate of the glucan synthesis reaction.
8. The method of embodiment 5, wherein the glucan synthesis reaction produces at least one soluble alpha-glucan product that is
  - (i) a product of a glucosyltransferase, or
  - (ii) a product of the concerted action of both a glucosyltransferase and an alpha-glucanohydrolase capable of hydrolyzing glucan polymers having one or more alpha-1,3-glycosidic linkages or one or more alpha-1,6-glycosidic linkages.
9. The method of embodiment 8, wherein the fraction is a chromatographic fraction of the glucan synthesis reaction.
10. The method of any one of embodiments 1-9, wherein the alpha-glucosidase enzyme is a transglucosidase.
11. A composition produced by contacting a saccharide with an alpha-glucosidase enzyme, wherein the saccharide is a disaccharide or oligosaccharide and comprises at least one alpha-1,3 or alpha-1,6 glucosyl-glucose linkage, wherein the enzyme hydrolyzes at least one alpha-1,3 or alpha-1,6 glucosyl-glucose linkage of the saccharide, and wherein the composition comprises a reduced amount of the saccharide compared to the amount of the saccharide that was present prior to the contacting.
12. The composition of embodiment 11, wherein the degree of polymerization of the saccharide before hydrolysis is 3 to 7.
13. The composition of embodiment 11 or 12, wherein the saccharide is in (i) a glucan synthesis reaction, or (ii) a fraction obtained from the glucan synthesis reaction;

- wherein the saccharide is a byproduct of the glucan synthesis reaction.
14. A method of enriching fructose present in a fraction of a glucan synthesis reaction, comprising:
- contacting a fraction obtained from a glucan synthesis reaction with an alpha-glucosidase enzyme under suitable conditions, wherein the alpha-glucosidase enzyme hydrolyzes at least one alpha-1,3 or alpha-1,6 glucosyl-glucose linkage of a disaccharide or oligosaccharide comprised within the fraction; and
  - separating fructose from the hydrolyzed fraction of step (a) to obtain a composition having a higher concentration of fructose compared to the fructose concentration of the fraction of step (a).
15. A fermentation method comprising:
- contacting a fraction obtained from a glucan synthesis reaction with an alpha-glucosidase enzyme under suitable conditions, wherein the alpha-glucosidase enzyme hydrolyzes at least one alpha-1,3 or alpha-1,6 glucosyl-glucose linkage of a disaccharide or oligosaccharide comprised within the fraction;
  - fermenting the fraction of step (a) with a microbe to yield a product, wherein the fermenting is performed after step (a) or simultaneously with step (a); and
  - optionally, isolating the product of (b);
- wherein the yield of the product of (b) is increased compared to the product yield of fermenting a fraction of the glucan synthesis reaction that has not been contacted with the alpha-glucosidase enzyme.

### EXAMPLES

The disclosed invention is further defined in the following Examples. It should be understood that these Examples, while indicating certain preferred aspects of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various uses and conditions.

#### Abbreviations

The meaning of some of the abbreviations used herein is as follows: "g" means gram(s), "h" means hour(s), "mL" means milliliter(s), "psi" means pound(s) per square inch, "wt %" means weight percentage, "μm" means micrometer(s), "%" means percent, "° C." means degrees Celsius, "mg" means milligram(s), "mm" means millimeter(s), "mL/min" means milliliters per minute, "m" means meter(s), "μL" means microliter(s), "mmol" means millimole(s), "min" means minute(s), "mol %" means mole percent, "M" means molar, "mg/g" means milligram per gram, "rpm" means revolutions per minute, "MPa" means megapascals.

#### General Methods

All reagents were obtained from Sigma-Aldrich (St. Louis, Mo.) unless stated otherwise. Sucrose was obtained from VWR (Radnor, Pa.).

#### Preparation of Crude Extracts of Glucosyltransferase (gtf) Enzymes

The *Streptococcus salivarius* gtfJ enzyme (SEQ ID NO:3) was expressed in *E. coli* strain DH10B using an isopropyl beta-D-1-thiogalactopyranoside (IPTG)-induced expression system. SEQ ID NO:3 has an N-terminal 42-residue deletion compared to the *S. salivarius* gtfJ amino acid sequence in GENBANK Identification No. 47527, but includes a start methionine. Briefly, *E. coli* DH10B cells were transformed

to express SEQ ID NO:3 from a DNA sequence codon-optimized to express the gtfJ enzyme in *E. coli*. This DNA sequence was contained in the expression vector, pJexpress404® (DNA 2.0, Menlo Park Calif.). The transformed cells were inoculated to an initial optical density (OD at 600<sub>nm</sub>) of 0.025 in LB medium (10 g/L Tryptone; 5 g/L yeast extract, 10 g/L NaCl) and allowed to grow at 37° C. in an incubator while shaking at 250 rpm. The cultures were induced by addition of 1 mM IPTG when they reached an OD<sub>600</sub> of 0.8-1.0. Induced cultures were left on the shaker and harvested 3 hours post induction.

GtfJ enzyme (SEQ ID NO:3) was harvested by centrifuging cultured cells (25° C., 16000 rpm) in an Eppendorf® centrifuge, re-suspending the cells in 5.0 mM phosphate buffer (pH 7.0) and cooling to 4° C. on ice. The cells were broken using a bead beater with 0.1-mm silica beads, and then centrifuged at 16000 rpm at 4° C. to pellet the unbroken cells and cell debris. The crude extract (containing soluble GtfJ enzyme, SEQ ID NO:3) was separated from the pellet and analyzed by Bradford protein assay to determine protein concentration (mg/mL).

The *Streptococcus* sp. C150 gtf-S enzyme (SEQ ID NO:40) was prepared as follows. SG1184 is a *Bacillus subtilis* expression strain that expresses a truncated version of the glycosyltransferase Gtf-S ("GTF0459") from *Streptococcus* sp. C150 (GENBANK® GI:321278321). The gene (SEQ ID NO:41) encoding an N-terminal truncated protein GTF0459 (SEQ ID NO:42) from *E. coli* expression plasmid pMP79 was cloned into the NheI and HindIII sites of the *Bacillus subtilis* integrative expression plasmid p4JH under the aprE promoter and fused with the *B. subtilis* AprE signal peptide on the vector. The construct was first transformed into *E. coli* DH10B and selected on LB with ampicillin (100 μg/mL) plates. The confirmed construct pDCQ984 expressing GTF0459 was then transformed into *B. subtilis* BG6006 containing nine protease deletions (amyE::xyIRPxy-lAcomK-ermC, degUHy32, oppA, AspOIE3501, ΔaprE, ΔnprE, Δepr, ΔispA, Δbpr, Δvpr, ΔwprA, Δmpr-ybJ, ΔnprB) and selected on LB plates with chloramphenicol (5 μg/mL). The colonies grown on LB plates with 5 μg/mL chloramphenicol were streaked several times onto LB plates with 25 μg/mL chloramphenicol. The resulting *B. subtilis* expression strain, SG1184, was first grown in LB medium with 25 μg/mL chloramphenicol and then subcultured into GrantsII medium containing 25 μg/mL chloramphenicol grown at 30° C. for 2-3 days. The cultures were spun at 15,000 g for 30 min at 4° C. and the supernatant was filtered through 0.22-μm filters. The filtered supernatant was aliquoted and frozen at -80° C.

*B. subtilis* SG1184 strain, expressing GTF0459 (SEQ ID NO:42), was grown under an aerobic submerged condition by conventional fed-batch fermentation. A nutrient medium was used containing 0-0.25% corn steep solids (Roquette), 5-25 g/L sodium and potassium phosphate, a solution of 0.3-0.6 M ferrous sulfate, manganese chloride and calcium chloride, 0.5-4 g/L magnesium sulfate, and a solution of 0.01-3.7 g/L zinc sulfate, cuprous sulfate, boric acid and citric acid. An antifoam agent, FOAMBLAST 882, at 2-4 mL/L was added to control foaming. A 10-L fermentation was fed with 50% (w/w) glucose feed when initial glucose in batch was non-detectable. The glucose feed rate was ramped over several hours. The fermentation was controlled at 30° C. and 20% DO, and at initial agitation of 750 rpm. The pH was controlled at 7.2 using 50% (v/v) ammonium hydroxide. Fermentation parameters such as pH, temperature, airflow, and DO % were monitored throughout the entire 2-day fermentation run. The culture broth was har-

vested at the end of the run and centrifuged to obtain supernatant. The supernatant containing GTF0459 (SEQ ID NO:42) was then stored frozen at  $-80^{\circ}\text{C}$ .

The *S. mutans* MT-4239 gtf-C enzyme (SEQ ID NO:43) was prepared as follows. A gene encoding a truncated version of a glucosyltransferase (gtf) enzyme identified in GENBANK® as GI:3130088 (SEQ ID NO:43; gtf-C from *S. mutans* MT-4239) was synthesized using codons optimized for expression in *Bacillus subtilis* and synthesized by GenScript. The gene (SEQ ID NO:44) encoding GTF0088BsT1 with an N-terminal truncation and a C-terminal T1 truncation (SEQ ID NO:45) was amplified from the GENSCRIPT plasmid and cloned into the NheI and HindIII sites of the *Bacillus subtilis* integrative expression plasmid p4JH under the aprE promoter and fused with the *B. subtilis* AprE signal peptide on the vector. The construct was first transformed into *E. coli* DH10B and selected on LB with ampicillin (100  $\mu\text{g}/\text{mL}$ ) plates. The confirmed construct pDCQ1021 expressing GTF0088BsT1 was then transformed into *B. subtilis* BG6006 containing nine protease deletions (amyE::xyIR-PxylAcomK-ermC, degUHy32, oppA,  $\Delta$ spolE3501,  $\Delta$ aprE,  $\Delta$ nprE,  $\Delta$ epr,  $\Delta$ ispA,  $\Delta$ bpr,  $\Delta$ vpr,  $\Delta$ wprA,  $\Delta$ mpr-yb1J,  $\Delta$ nprB) and selected on the LB plates with chloramphenicol (5  $\mu\text{g}/\text{mL}$ ). The colonies grown on LB plates with 5  $\mu\text{g}/\text{mL}$  chloramphenicol were streaked several times onto LB plates with 25  $\mu\text{g}/\text{mL}$  chloramphenicol. The resulting *B. subtilis* expression strain SG1221 was first grown in LB medium with 25  $\mu\text{g}/\text{mL}$  chloramphenicol and then subcultured into GrantsII medium containing 25  $\mu\text{g}/\text{mL}$  chloramphenicol grown at  $30^{\circ}\text{C}$ . for 2-3 days. The cultures were spun at 15,000 g for 30 min at  $4^{\circ}\text{C}$ . and the supernatant was filtered through 0.22- $\mu\text{m}$  filters. The filtered supernatant was aliquoted and frozen at  $-80^{\circ}\text{C}$ .

*B. subtilis* SG1221 strain, expressing GTF0088BsT1 (SEQ ID NO:45), was grown under an aerobic submerged condition by conventional fed-batch fermentation. A nutrient medium was used containing 0-0.25% corn steep solids (Roquette), 5-25 g/L sodium and potassium phosphate, a solution of 0.3-0.6 M ferrous sulfate, manganese chloride and calcium chloride, 0.5-4 g/L magnesium sulfate, and a solution of 0.01-3.7 g/L zinc sulfate, cuprous sulfate, boric acid and citric acid. An antifoam agent, FOAMBLAST 882, at 2-4 mL/L was added to control foaming. A 2-L fermentation was fed with 50% (w/w) glucose feed when initial glucose in batch was non-detectable. The glucose feed rate was ramped over several hours. The fermentation was controlled at  $30^{\circ}\text{C}$ . and 20% DO, and at an initial agitation of 400 rpm. The pH was controlled at 7.2 using 50% (v/v) ammonium hydroxide. Fermentation parameters such as pH, temperature, airflow, and DO % were monitored throughout the entire 2-day fermentation run. The culture broth was harvested at the end of run and centrifuged to obtain supernatant. The supernatant containing GTF088BsT1 (SEQ ID NO:45) was then stored frozen at  $-80^{\circ}\text{C}$ .

Determination of the Glucosyltransferase GTF0459 and GTF0088BsT1 Activity

Glucosyltransferase activity assay was performed by incubating 1-10% (v/v) crude protein extract containing GTF enzyme with 200 g/L sucrose in 25 mM or 50 mM sodium acetate buffer at pH 5.5 in the presence or absence of 25 g/L dextran (MW ~1500, Sigma-Aldrich, Cat.#31394) at  $37^{\circ}\text{C}$ . and 125 rpm orbital shaking. One aliquot of reaction mixture was withdrawn at 1 h, 2 h and 3 h and heated at  $90^{\circ}\text{C}$ . for 5 min to inactivate the GTF. The insoluble material was removed by centrifugation at 13,000 $\times$ g for 5 min, followed by filtration through 0.2- $\mu\text{m}$  RC (regenerated cellulose) membrane. The resulting filtrate

was analyzed by HPLC using two AMINEX HPX-87C columns series at  $85^{\circ}\text{C}$ . (BioRad, Hercules, Calif.) to quantify sucrose concentration. The sucrose concentration at each time point was plotted against the reaction time and the initial reaction rate was determined from the slope of the linear plot. One unit of GTF activity was defined as the amount of enzyme needed to consume one micromole of sucrose in one minute under the assay conditions.

Preparation of a Crude Extract of Alpha-(1,3)-Glucanohydrolase (Mutanase)

A gene encoding the *Penicillium marneffe* ATCC® 18224™ mutanase identified in GENBANK® as GI:212533325 was synthesized by GenScript (Piscataway, N.J.). The nucleotide sequence (SEQ ID NO:46) encoding protein sequence (MUT3325; SEQ ID NO:47) was sub-cloned into plasmid pTrex3 at SacII and AscI restriction sites, a vector designed to express the gene of interest in *Trichoderma reesei*, under control of CBHI promoter and terminator, with *Aspergillus niger* acetamidase for selection. The resulting plasmid was transformed into *T. reesei* by biolistic injection. The detailed method of biolistic transformation is described in International PCT Patent Application Publication WO2009/126773 A1, which is incorporated herein by reference. A 1-cm<sup>2</sup> agar plug with spores from a stable clone, TRM05-3, was used to inoculate the production media (described below). The culture was grown in shake flasks for 4-5 days at  $28^{\circ}\text{C}$ . and 220 rpm. To harvest the secreted proteins, the cell mass was first removed by centrifugation at 4000 g for 10 min and the supernatant was filtered through 0.2- $\mu\text{m}$  sterile filters. The expression of mutanase MUT3325 (SEQ ID NO:47) was confirmed by SDS-PAGE.

The production media component is listed below. NREL-Trich Lactose Defined

Formula	Amount	Units
ammonium sulfate	5	g
PIPPS	33	g
BD BACTO casamino acid	9	g
KH <sub>2</sub> PO <sub>4</sub>	4.5	g
CaCl <sub>2</sub> •2H <sub>2</sub> O	1.32	g
MgSO <sub>4</sub> •7H <sub>2</sub> O	1	g
<i>T. reesei</i> trace elements	2.5	mL
NaOH pellet	4.25	g
Adjust pH to 5.5 with 50% NaOH		
Bring volume to	920	mL
Add to each aliquot: FOAMBLAST	5	drops
Autoclave, then add 20% lactose filter sterilized	80	mL

#### *T. reesei* Trace Elements

Formula	Amount	Units
citric acid•H <sub>2</sub> O	191.41	g
FeSO <sub>4</sub> •7H <sub>2</sub> O	200	g
ZnSO <sub>4</sub> •7H <sub>2</sub> O	16	g
CuSO <sub>4</sub> •5H <sub>2</sub> O	3.2	g
MnSO <sub>4</sub> •H <sub>2</sub> O	1.4	g
H <sub>3</sub> BO <sub>3</sub> (boric acid)	0.8	g
Bring volume to	1	L

Fermentation seed culture was prepared by inoculating 0.5 L of minimal medium in a 2-L baffled flask with 1.0 mL frozen spore suspension of the MUT3325 expression strain TRM05-3 (The minimal medium was composed of 5 g/L

ammonium sulfate, 4.5 g/L potassium phosphate monobasic, 1.0 g/L magnesium sulfate heptahydrate, 14.4 g/L citric acid anhydrous, 1 g/L calcium chloride dihydrate, 25 g/L glucose and trace elements including 0.4375 g/L citric acid, 0.5 g/L ferrous sulfate heptahydrate, 0.04 g/L zinc sulfate heptahydrate, 0.008 g/L cupric sulfate pentahydrate, 0.0035 g/L manganese sulfate monohydrate and 0.002 g/L boric acid. The pH was 5.5.). The culture was grown at 32° C. and 170 rpm for 48 hours before being transferred to 8 L of the production medium in a 14-L fermenter. The production medium was composed of 75 g/L glucose, 4.5 g/L potassium phosphate monobasic, 0.6 g/L calcium chloride dehydrate, 1.0 g/L magnesium sulfate heptahydrate, 7.0 g/L ammonium sulfate, 0.5 g/L citric acid anhydrous, 0.5 g/L ferrous sulfate heptahydrate, 0.04 g/L zinc sulfate heptahydrate, 0.00175 g/L cupric sulfate pentahydrate, 0.0035 g/L manganese sulfate monohydrate, 0.002 g/L boric acid and 0.3 mL/L FOAMBLAST 882.

The fermentation was first run with batch growth on glucose at 34° C., 500 rpm for 24 h. At the end of 24 h, the temperature was lowered to 28° C. and the agitation speed was increased to 1000 rpm. The fermenter was then fed with a mixture of glucose and sophorose (62% w/w) at a specific feed rate of 0.030 g glucose-sophorose solids/g biomass/hr. At the end of run, the biomass was removed by centrifugation and the supernatant containing the MUT3325 mutanase (SEQ ID NO:47) was concentrated about 10-fold by ultrafiltration using 10-kD Molecular Weight Cut-Off ultrafiltration cartridge (UFP-10-E-35; GE Healthcare, Little Chalfont, Buckinghamshire, UK). The concentrated protein was stored at -80° C.

#### Determination of Alpha-Glucanohydrolase (Mutanase) Activity

Insoluble mutan polymers required for determining mutanase activity were prepared using secreted enzymes produced by *Streptococcus sobrinus* ATCC® 33478™. Specifically, one loop of glycerol stock of *S. sobrinus* ATCC® 33478™ was streaked on a BHI agar plate (Brain Heart Infusion agar, Teknova, Hollister, Calif.), and the plate was incubated at 37° C. for 2 days. A few colonies were picked using a loop to inoculate 2x100 mL BHI liquid medium in the original medium bottle from Teknova, and the culture was incubated at 37° C., held static for 24 h. The resulting cells were removed by centrifugation and the resulting supernatant was filtered through a 0.2-µm sterile filter; 2x101 mL of filtrate was collected. To the filtrate was added 2x11.2 mL of 200 g/L sucrose (final sucrose 20 g/L). The reaction was incubated at 37° C. with no agitation for 67 h. The resulting polysaccharide polymers were collected by centrifugation at 5000xg for 10 min. The supernatant was carefully decanted. The insoluble polymers were washed 4 times with 40 mL of sterile water. The resulting mutan polymers were lyophilized for 48 h. Mutan polymer (390 mg) was suspended in 39 mL of sterile water to make a 10 mg/mL suspension. The mutan suspension was homogenized by sonication (40% amplitude until large lumps disappear, ~10 min in total). The homogenized suspension was aliquoted and stored at 4° C.

A mutanase assay was initiated by incubating an appropriate amount of enzyme with 0.5 mg/mL mutan polymer (prepared as described above) in 25 mM KOAc buffer at pH 5.5 and 37° C. At various time points, an aliquot of reaction mixture was withdrawn and quenched with equal volume of 100 mM glycine buffer (pH 10). The insoluble material in each quenched sample was removed by centrifugation at 14,000xg for 5 min. The reducing ends of oligosaccharide and polysaccharide polymer produced at each time point

were quantified by the p-hydroxybenzoic acid hydrazide solution (PAHBAH) assay (Lever M., *Anal. Biochem.*, (1972) 47:273-279) and the initial rate was determined from the slope of the linear plot of the first three or four time points of the time course. The PAHBAH assay was performed by adding 10 µL of reaction sample supernatant to 100 µL of PAHBAH working solution and heated at 95° C. for 5 min. The working solution was prepared by mixing one part of reagent A (0.05 g/mL p-hydroxy benzoic acid hydrazide and 5% by volume of concentrated hydrochloric acid) and four parts of reagent B (0.05 g/mL NaOH, 0.2 g/mL sodium potassium tartrate). The absorption at 410 nm was recorded and the concentration of the reducing ends was calculated by subtracting appropriate background absorption and using a standard curve generated with various concentrations of glucose as standards.

#### Analysis of Reaction Profiles by HPLC

Periodic samples from reactions were taken and analyzed using an Agilent® 1260 HPLC equipped with a refractive index detector. An Aminex® HP-87C column (BioRad, Hercules, Calif.) having deionized water at a flow rate of 0.6 mL/min and 85° C. was used to quantitate the level of sucrose, glucose, leucrose and fructose in gtf reactions. An Aminex® HP-42A column (BioRad) having deionized water at a flow rate of 0.6 mL/min and 85° C. was used to quantitate soluble oligosaccharide byproducts (DP2-DP7) in gtf reactions.

A Dionex® UltiMate™ 3000 HPLC (Thermo Scientific) equipped with a refractive index detector was used for samples involving immobilized enzymes (Example 4). A Phenomenex® Rezex™ calcium monosaccharide column having deionized water at a flow rate of 0.3 mL/min and 85° C. was used to analyze the sugars.

#### Analysis of Oligosaccharide Linkage by NMR

NMR data were acquired on an Agilent DD2 spectrometer operating at 500 MHz for <sup>1</sup>H using a 5-mm cryogenic triple-resonance pulsed-field gradient (PFG) probe. Water suppression was obtained by carefully placing the observe transmitter frequency on resonance for the residual water signal in a "presat" experiment, and then using the first slice of a NOESY experiment with a full phase cycle (multiple of 32) and a mix time of 10 ms. One-dimensional <sup>1</sup>H spectra were acquired with a spectral width of 6410 Hz, acquisition time of 5.1 s, 65536 data points, 4 s presaturation and a 90-degree pulse of 5.85 µs. Sample temperature was maintained at 25° C. Samples were prepared by adding 50 µL to a 5-mm NMR tube along with 450 µL of D<sub>2</sub>O and 60 µL of D<sub>2</sub>O containing 12.4 mM DSS (4,4-dimethyl-4-silapentane-1-sulfonic acid sodium salt) internal reference with the methyl resonance set to 0 ppm. Chemical shift assignments for different anomeric linkages were taken from: Goffin et al. (2009, *Bull Korean Chem. Soc.* 30:2535-2541). Peak assignments were 5.35 ppm for alpha(1,3) linkages, 5.1 ppm for leucrose, and 4.95 for alpha(1,6) linkages. Reducing ends (RE) were assigned to be 5.2 for alpha RE and 4.65 for beta RE.

#### Example 1

##### Production of Sugar Syrup by Polymerization of Sucrose

This example discloses the general manner in which a mixture of soluble sugars was produced by polymerization of sucrose with a gtf enzyme in a glucan synthesis reaction. Specifically, a filtrate of a glucan synthesis reaction was prepared, which was then concentrated to a syrup.

37

Sucrose (3000 g) was added to a clean 5-gallon polyethylene bucket. Water (18.1 L) and Fermasure™ (10 mL) were added to the bucket, and the pH was adjusted to 7.0 by addition of 5 vol % NaOH and 5 vol % H<sub>2</sub>SO<sub>4</sub>. The final volume was ~20 L and the initial concentration of sucrose as measured by HPLC was 152.5 g/L. The glucan polymerization reaction was initiated by adding 0.3 vol % of crude gtf enzyme (SEQ ID NO:3) extract prepared as described in the General Methods section. This extract contained about 2.9 mg/mL of protein. Agitation to the reaction solution was provided using an overhead mechanical motor equipped with a glass shaft and PTFE blade.

After 48 hours, HPLC analysis revealed that 96% of the sucrose had been consumed and the reaction was deemed to be complete. The insoluble poly-alpha-1,3-glucan product of the reaction was removed by filtration with a Buchner filter funnel using 325-mesh steel screen and 40-micron filter paper. The mother liquor (filtrate) was then concentrated using a rotary evaporator (bath temp of 40-50° C.) to a total sugar concentration of about 320 g/L sugars. The composition of the concentrated filtrate is provided in Table 2.

TABLE 2

Composition of a Concentrated Filtrate of a Glucan Synthesis Reaction							
	Sucrose	Leucrose	Glucose	Fructose	DP2	DP3+	Total
g/L	13.5	130.6	25.5	103.8	18.3	28.3	320.1
wt %	4.2	40.8	8	32.4	5.7	8.9	100

Table 2 indicates that the concentrated filtrate of the glucan synthesis reaction contains sucrose, fructose, glucose, leucrose and oligosaccharides of DP2-DP7.

## Example 2

## Effect of Enzymes on Hydrolysis of Sugars in a Filtrate of a Glucan Synthesis Reaction

This example measures the activity of various glucoamylase (EC 3.2.1.3), transglucosidase (EC 2.4.1.24), beta-

38

glucosidase (EC 3.2.1.21), alpha-amylase (EC 3.2.1.1) and glucosidase (EC 3.2.1) enzymes for the purpose of reducing the concentration of leucrose and/or oligosaccharide byproducts in a concentrated filtrate of a glucan synthesis reaction. Certain enzymes such as DIAZYME RDF ULTRA, transglucosidase (EC 2.4.1.24) and glucoamylase (EC 3.2.1.3), which are all alpha-glucosidase, were found to be particularly effective at reducing the amount of these byproducts, resulting in a corresponding increase in monosaccharides (glucose and fructose) in the treated filtrate.

A filtrate of a glucan synthesis reaction was first prepared and concentrated to a syrup according to the procedure outline in Example 1. The composition of this concentrated filtrate is provided in Table 3. NMR analysis revealed that the ratio of alpha(1,3) to alpha (1,6) linkages present in the syrup was 78:22.

TABLE 3

Composition of a Concentrated Filtrate of a Glucan Synthesis Reaction							
	Sucrose	Leucrose	Glucose	Fructose	DP2	DP3+	Total
g/L	161	210	93	302	33	61	860
wt %	18.7	24.4	10.8	35.1	3.8	7.1	100.0

The syrup of Table 3 was used to test the hydrolytic activity of various enzymes against leucrose and oligosaccharide byproducts of the glucan synthesis reaction. It was not obvious at the outset of these experiments what enzyme could be used to hydrolyze both these byproducts, given that leucrose contains an unusual linkage [alpha(1,5)-glucosyl fructose] and that the oligosaccharides comprise primarily alpha(1,3) and alpha(1,6) glucosyl-glucose linkages. Enzymes with various activities were selected for this analysis (Table 4).

TABLE 4

Enzymes Evaluated for Leucrose and Oligosaccharide Hydrolysis			
Enzyme	Source	Function	Activity or protein concentration
DIAZYME RDF ULTRA	DuPont IB <sup>®</sup>	1,4-alpha-glucosidase	710 U/g
Oligo-1,6-glucosidase	Megazyme	1,6-alpha-glucosidase	320 U/mg
SPEZYME FRED	DuPont IB	Alpha-amylase	1-5%
SPEZYME RSL	DuPont IB	Alpha-amylase	1-5%
OPTIMAX L-1000	DuPont IB	Pullulanase	1-5%
TRANSGLUCOSIDASE L-2000	DuPont IB	Transglucosidase	>1700 TGU/g
purified TRANSGLUCOSIDASE L-2000	DuPont IB	Transglucosidase	22.7 mg/mL
ACCELLERASE BG	DuPont IB	Beta-glucosidase	3000 U/g
NOVO 188	Sigma-Aldrich	Beta-glucosidase	>250 U/g
SUMIZYME BFS-L	Shin Nihon Chemical	Beta-glucosidase	100 U/g
SUMIZYME BGA	Shin Nihon Chemical	Beta-glucosidase	2000 U/g
ACCELLERASE TRIO	DuPont IB	Cellulase	5-10%
ACCELLERASE 1500	DuPont IB	Cellulase/Beta-glucosidase	5-10%/0.5-4%
OPTIDEX L-400	DuPont IB	Glucoamylase	>350 GAU/g

TABLE 4-continued

Enzymes Evaluated for Leucrose and Oligosaccharide Hydrolysis			
Enzyme	Source	Function	Activity or protein concentration
GC 147	DuPont IB	Glucoamylase	400 GAU/g
GC 321	DuPont IB	Glucoamylase	>350 GAU/g

<sup>a</sup>DuPont Industrial Biosciences

Conditions for treating the syrup of Table 3 with each of the above enzymes are provided in Table 5 (enzyme loading, time, temperature, pH, sugar concentration). The syrup was diluted with water to reach the sugar concentration used in each hydrolysis reaction. Table 5 further provides the percent hydrolysis of the leucrose and DP3+(at least DP3-DP7) oligosaccharides by each enzyme. Percent DP3+ hydrolysis was calculated as  $(1 - (\text{wt \% DP3+ oligosaccharides in the final syrup}) / (\text{wt \% DP3+ oligosaccharides in the initial syrup}))$ . Similarly, percent leucrose hydrolysis was calculated as  $(1 - (\text{wt \% leucrose in the final syrup}) / (\text{wt \% leucrose in the initial syrup}))$ .

Cellulases (Examples 2.14 and 2.15) were largely ineffective at hydrolyzing leucrose, but did hydrolyze some of the oligosaccharides.

Although the oligosaccharides did not contain beta linkages, surprisingly, beta-glucosidase enzymes also showed a range of hydrolytic conversion from very low (ACCELERASE BG, Example 2.9) to very high (NOVO 188, Examples 2.10 and 2.11). The relative efficacy of these enzymes varied quite dramatically. In some cases, the amount of oligosaccharide that was hydrolyzed greatly exceeded (Example 2.11), or was close to (Example 2.12), the percentage of leucrose that was hydrolyzed. In other

TABLE 5

Hydrolysis of Leucrose and Oligosaccharides in a Concentrated Filtrate by Various Enzymes								
Example	Enzyme	Enzyme loading (vol %)	Temp (° C.)	Time (hr)	pH	Sugar concentration (g/L) <sup>a</sup>	DP3+ <sup>b</sup> hydrolysis (%)	Leucrose hydrolysis (%)
2.1	DIAZYME RDF ULTRA	0.5	60	88	4.0	300	36	13
2.2	Oligo-1,6-glucosidase	5	40	72	5.5	400	43	<2
2.3	SPEZYME FRED	0.5	60	66	4.0	280	<2	<2
2.4	SPEZYME RSL	0.5	60	48	4	290	<2	<2
2.5	OPTIMAX L-1000	0.5	60	48	4	290	<2	<2
2.6	TG L-2000	0.25	60	70	4.0	260	54	>98
2.7	TG L-2000	2	60	48	4.5	300	96	>98
2.8	PURIFIED TG L-2000	0.5	60	48	4.5	260	56	>98
2.9	ACCELERASE BG	0.5	60	70	4	300	11	<2
2.10	NOVO 188	0.25	60	70	4	300	49	36
2.11	NOVO 188	5	60	40	5.5	340	93	29
2.12	SUMIZYME BFS-L	0.5	60	48	4.5	260	55	46
2.13	SUMIZYME BGA	0.1 wt %	60	48	4.5	260	26	77
2.14	ACCELERASE TRIO	0.5	60	48	4	290	28	6.6
2.15	ACCELERASE 1500	0.5	60	66	4	280	26	4
2.16	GC 147	0.5	60	40	4	300	55	12
2.17	GC 321	5	60	72	5.5	400	74	64
2.18	OPTIDEX L-400	0.5	60	70	4	300	27	25

<sup>a</sup>Sugar concentration (total concentration of sucrose, glucose, fructose, leucrose and oligosaccharides) measured by HPLC; reported values are rounded to nearest 10 g/L increment.

<sup>b</sup>DP3+ contains DP3-DP7, but may also contain larger soluble oligosaccharides that have a high ratio of alpha-1,6 linkages to alpha-1,3 linkages, when produced using certain gtf enzymes.

Table 5 indicates that 1,4-alpha-glucosidase and 1,6-alpha-glucosidase showed some (Example 2.1) or very little (Example 2.2) hydrolysis of leucrose, but did release some glucose from the oligosaccharides. Use of alpha-amylase (Example 2.3 and Example 2.4) showed very little activity against the compounds of interest. Similarly, use of a pululanase (Example 2.5) showed very little activity.

cases, leucrose was highly hydrolyzed by beta-glucosidase while the oligosaccharides were moderately hydrolyzed (Example 2.13). The high disparity amongst the results observed with beta-glucosidase suggests that the presence of other enzymes in the tested beta-glucosidase formulations, such as glucoamylase or another type of alpha-glucosidase, could be responsible for the observed activity.

Conversely, the results in Table 5 indicate that transglucosidase (TG L-2000, Example 2.6) showed very high activity at hydrolyzing both the oligosaccharides and leucrose. Leucrose hydrolysis by transglucosidase appeared quantitative under certain circumstances, and greater than 95% of the DP3+ material was hydrolyzed to glucose and DP2 at high enzyme loadings (Example 2.7). Use of a purified version of transglucosidase revealed similar activity (Example 2.8), indicating that the observed hydrolysis is due to the transglucosidase enzyme and not background activity.

Glucoamylases (Examples 2.16-2.18) showed a range of activity against leucrose and the oligosaccharides. Only one tested glucoamylase (Example 2.18) gave less than 30% hydrolysis of both the leucrose and oligosaccharides.

The results in Table 5 indicate that alpha-glucosidases such as DIAZYME RDF ULTRA, glucoamylase and transglucosidase can hydrolyze leucrose byproduct present in a glucan reaction filtrate. The ability of alpha-glucosidases to hydrolyze leucrose indicates that these enzymes can hydrolyze alpha-1,5 glucosyl-fructose linkages. While this activity was shown above using leucrose as a substrate, it is believed that this activity can also be extended to oligosaccharides comprising alpha-1,5 glucosyl-fructose linkages.

The results in Table 5 further indicate that alpha-glucosidases such as glucoamylase and transglucosidase can hydrolyze oligosaccharide byproducts present in a glucan reaction filtrate. Since these oligosaccharides are mostly comprised of glucose monomer units linked by alpha-1,3 and/or alpha-1,6 linkages (Example 3), the data in Table 5 indicate that alpha-glucosidase enzymes can hydrolyze alpha-1,3 glucosyl-glucose and/or alpha-1,6 glucosyl-glucose linkages.

Since alpha-glucosidase enzymes were generally effective at hydrolyzing the leucrose and/or oligosaccharide byproducts of a glucan synthesis reaction, these enzymes can be used alone or in combination to reduce the processing time necessary to generate a high purity syrup from a glucan reaction filtrate containing an increased amount of monosaccharides and reduced amount of sugar byproducts. An example of an effective enzyme combination could be a transglucosidase such as TG L-2000, for leucrose hydrolysis, and a glucoamylase (e.g., GC 321) enzyme that efficiently hydrolyzes oligosaccharide byproducts.

Thus, alpha-glucosidase enzymes can individually hydrolyze (i) alpha-1,5 glucosyl-fructose linkages and (ii) alpha-1,3 and alpha-1,6 glucosyl-glucose linkages in certain saccharides.

### Example 3

#### Comparison of Linkage Distributions of Glucan Reaction Filtrate Components Before and after Enzyme Hydrolysis

This example measures the hydrolytic activity of transglucosidase (EC 2.4.1.24) and beta-glucosidase (EC 3.2.1.21) enzymes against leucrose and oligosaccharide byproducts present in a concentrated filtrate of a glucan synthesis reaction. Transglucosidase was found to reduce the amount of these byproducts, resulting in a corresponding increase in monosaccharides (glucose and fructose) in the treated filtrate.

The oligosaccharide byproducts present in the filtrate of the above glucan synthesis reaction comprise >90% glucose-glucose linkages, as determined by NMR (General Methods). Of the glucose-glucose linkages, ~78% represent alpha-1,3 linkages and ~22% represent alpha-1,6 linkages.

NMR was used to determine the linkage profile of material generated in Example 2.11 above after hydrolysis. As shown in FIG. 1, the peak corresponding to alpha-1,3 linkages was reduced by 86%, the peak corresponding to alpha-1,6 linkages was reduced by only 2.3%, and the peak corresponding to leucrose peaks was reduced by 21%. While sucrose was very nearly quantitatively hydrolyzed by this enzyme, Novo 188 does not appear to be capable of hydrolyzing alpha-1,6 linkages.

NMR was similarly used to determine the linkage profile of material generated using TG L-2000 (SEQ ID NO:1) transglucosidase (FIG. 2). 210  $\mu$ L of concentrated filtrate from the material in Table 3, 300  $\mu$ L of D<sub>2</sub>O, and 90  $\mu$ L of D<sub>2</sub>O containing 12.4 mM DSS as internal reference were mixed in an NMR tube to give a total sugar concentration of 300 g/L and heated to 60° C. A time zero spectrum (starting material in FIG. 2) was acquired after thermal equilibration at 60° C., and then 0.5 vol % of enzyme was added. The sample was re-equilibrated in the probe at 60° C. and shimmed, and measurements were taken within a few minutes of analysis. After 10 hours of treatment with TG L-2000 enzyme (treated material in FIG. 2), the peaks corresponding to alpha-1,3 linkages were reduced by 41%, the peaks corresponding to alpha-1,6 linkages were reduced by 36%, and the peak corresponding to leucrose was reduced by >95% (FIG. 2). An increase in both the alpha-reducing end and beta-reducing end peaks was observed, which corresponds to an increase in fructose and glucose (FIG. 2).

These results demonstrate that a transglucosidase can convert oligosaccharides containing alpha-1,3 and alpha-1,6 linkages into glucose and can convert leucrose into fructose and glucose. Thus, transglucosidase can hydrolyze (i) alpha-1,5 glucosyl-fructose linkages and (ii) alpha-1,3 and alpha-1,6 glucosyl-glucose linkages in certain saccharides.

### Example 4

#### Hydrolysis of Leucrose and Oligosaccharides in Glucan Reaction Filtrate Using Immobilized Enzymes

This Example describes using immobilized glucoamylase (EC 3.2.1.3) and transglucosidase (EC 2.4.1.24) to hydrolyze leucrose and other oligosaccharides present in filtrate obtained from a glucan synthesis reaction. Specifically, the effect of immobilized transglucosidase TG L-2000 (SEQ ID NO:1, obtained from Genencor/DuPont Industrial Biosciences) and immobilized glucoamylase GC-147 (obtained from Genencor/DuPont Industrial Biosciences) on the hydrolysis of leucrose and oligosaccharides DP2, DP3 and HS (higher sugars, DP4+) in a filtrate of a glucan synthesis reaction was studied.

Immobilization of the glucoamylase and transglucosidase enzymes was carried out according to the method described in U.S. Pat. No. 5,541,097, which is incorporated herein by reference.

In a typical process for immobilizing the glucoamylase or transglucosidase, two batches of about 8.0 g/batch of porous granular diatomaceous earth (EP Minerals, Reno, Nev.) were hydrated with distilled water and then transferred to a glass column reactor of 1.5-cm diameter and 30-cm height. Water was pumped upflow at about 6-7 mL/min to remove fines from all three columns. Generally, within an hour the water effluent was free of fines. Water was drained from the column to the top of the granular diatomaceous earth beds and replaced with 0.1% w/v aqueous solution of polyethylenimine (PEI, EPOMIN P-1050). 3500 mL of the PEI

solution was then pumped upflow and effluent was recycled through the beds for 2 hours. The granular diatomaceous earth beds were then washed upflow with distilled water for 2 hours to remove free PEI at room temperature. In this manner, granular diatomaceous earth-PEI carriers were obtained.

In the meantime, 3.5 mL of glucoamylase GC-147 having activity defined in Table 4 was added to 315 ml of 0.02 M acetate buffer (pH 4.5). 1.575 g of 50% w/w glutaraldehyde (Protectol® GA-50) was then slowly added to the aqueous solution of glucoamylase with gentle mixing, and the glutaraldehyde was allowed to react with the aqueous glucoamylase solution for 4 hours at a temperature of 20-25° C. with gentle agitation, which resulted in formation of a treated enzyme-glutaraldehyde adduct containing treated glucoamylase. Separately, these steps were repeated using the transglucosidase TG L-2000 having activity defined in Table 4 instead of the glucoamylase, thereby resulting in the formation of a treated enzyme-glutaraldehyde adduct containing treated transglucosidase.

Each of the treated enzyme-glutaraldehyde adducts was then circulated for 4 hours (20-25° C.) in its own column prepared as above containing granular diatomaceous earth-PEI carrier. Excess treated adduct was then washed out of the carriers with water. Columns with immobilized glucoamylase or transglucosidase were thus prepared.

A glucan filtrate having the composition defined in Table 3 was diluted to 180 g/L, adjusted to pH 4.5, and passed through a column containing an immobilized enzyme. Column temperature was controlled to 60° C. After 16 hours of column equilibration, samples were taken periodically at different flow rates. Sugar compositions of hydrolysis reaction products were determined by HPLC (Table 6). Every time the flow rate setting was changed, the column was allowed to re-equilibrate for at least 1-2 bed volumes before sampling. The degree of hydrolysis of leucrose and oligosaccharides was calculated using the manner described in Example 2. Three column configurations were tested: 1) immobilized glucoamylase, 2) immobilized transglucosidase, and 3) immobilized glucoamylase followed by immobilized transglucosidase.

TABLE 6

Application of Immobilized Glucoamylase and Transglucosidase Enzymes to Hydrolyze Oligosaccharides and Leucrose			
Immobilized Enzyme	Mean contact time (hr)	DP3+ hydrolysis (%)	Leucrose hydrolysis (%)
GC 147	0.7	16	17
GC 147	1.0	20	22
GC 147	1.3	25	29
GC 147	3.0	39	47
TG L-2000	0.7	28	>95
TG L-2000	1.0	32	>95
TG L-2000	1.3	37	>95
TG L-2000	3.0	47	>95
GC 147 + TG L-2000	6.0	55	>95

Table 6 indicates that, as the mean contact time (defined as the nominal column volume divided by the mean flow rate) was increased, the degree of hydrolysis of leucrose and oligosaccharides generally increased. Use of the immobilized transglucosidase to hydrolyze leucrose was particularly effective, as no significant difference was observed even using the fastest flow rate that was tested. While each column individually showed reasonable conversion, the

combination of the glucoamylase and transglucosidase gave the highest hydrolysis of oligosaccharides.

Thus, use of an immobilized glucoamylase or transglucosidase, or both types of immobilized enzymes, represents an effective technique to hydrolyze oligosaccharides containing alpha-1,3 and alpha-1,6 glucosyl-glucose linkages, as well as leucrose. These results are consistent with those of Example 2. Immobilization of other alpha-glucosidase enzymes should give similar results.

Example 5

Enrichment of Fructose from a Glucan Reaction Filtrate by Chromatography

This example discloses how fructose in a glucan reaction filtrate can be further enriched through chromatography.

Generally, when separating sugar molecules by chromatography, components elute inversely to molecular size so that the largest molecules elute first. Thus, with respect to a filtrate of a glucan synthesis reaction, oligosaccharides elute first, followed by disaccharides, and then monosaccharides. Separations using a sodium cation resin did not separate fructose and glucose well, and all of leucrose, sucrose, and DP2 co-eluted. Use of ion exchange resins where the cation is calcium are preferred to separate glucose and fructose.

A filtrate of a glucan synthesis reaction was first prepared and concentrated to a syrup according to the procedure outline in Example 1. The composition of this concentrated filtrate is provided in Table 7.

TABLE 7

Composition of a Concentrated Filtrate of a Glucan Synthesis Reaction							
	Sucrose	Leucrose	Glucose	Fructose	DP2	DP3+	Total
g/L	126	202	93	295	40	65	821
wt %	15.4	24.6	11.3	36.0	4.8	7.9	100

The syrup of Table 7 was filtered and diluted to 25 g dry solids/100 g solution with ion-exchanged water, and fed to a column containing a crosslinked strong acid ion exchange resin in the calcium form. The physical parameters of this column appear in Table 8. Diluted syrup (15.8 L) was fed to the column, which was maintained at 65° C., after which the column was eluted using water at a flow rate of 30 L/hr.

TABLE 8

Physical Parameters of the Column	
Resin Type	FINEX CS11GC
Ion form	Ca <sup>2+</sup>
Crosslinking, % divinyl benzene	5.5
Particle size (mm)	0.34
Bed length (m)	5.0
Column diameter (m)	0.225

In this separation, leucrose remained in the column longer than sucrose, perhaps due to complexation of leucrose with the calcium cation, and in fact, co-eluted with glucose. Two fractions containing fructose were isolated. Fraction 5.1 eluted between 47 and 120 minutes, and fraction 5.2 eluted between 120 and 172 minutes. Of the fructose fed to the chromatographic separation, 95.7% of the fructose was isolated in >90% purity. The product distribution in each fraction (5.1 and 5.2) as measured by HPLC appears in Table 9.

45

TABLE 9

Product Distribution of Chromatographic Fractions Containing Significant Amounts of Fructose								
Frac- tion	Su- crose	Leucrose	Glu- cose	Fruc- tose	DP3+	Others	Total	% Fruc- tose recov- ered
5.1	31.9	34.8	20.8	3.9	5.4	4.8	100	3.9
5.2	0.0	1.0	0.8	97.7	0.0	0.6	100	95.7

As the feed composition for this separation comprised 36.0% fructose, a total of 34.5% of the total stream was recovered as a fructose syrup with >90 wt % DS fructose. If the sucrose in the feed is neglected, 40.7% of the sugars were recovered as a fructose syrup with >90 wt % DS fructose.

Thus, fructose in a glucan reaction filtrate can be further enriched through chromatography. Example 6 below demonstrates that this process can be enhanced using glucan filtrate hydrolyzed with a transglucosidase.

#### Example 6

##### Enrichment of Fructose from a Hydrolyzed Glucan Reaction Filtrate by Chromatography

This example demonstrates that fructose isolation from a glucan filtrate in which the oligosaccharides and leucrose have been hydrolyzed results in an increased yield of high purity fructose syrup compared to when isolating fructose from a non-hydrolyzed glucan filtrate.

A syrup was prepared by concentrating (vacuum at 50° C.) a glucan filtrate that had been treated with 1 vol % of transglucosidase TG L-2000 (SEQ ID NO:1) for 24 hr at 60° C. and pH 4.5. Some oligosaccharide formation was observed during the concentration process, which was expected since transglucosidase enzymes are known to create oligosaccharides at high concentrations of monosaccharides. The syrup had the final product distribution described in Table A.

TABLE A

Composition of a Concentrated Glucan Filtrate that Was Hydrolyzed before Concentration							
	Sucrose	Leucrose	Glucose	Fructose	DP2	DP3+	Total
g/L	3	<10	294	409	73	81	~870
wt %	0.3	1.1	33.7	47.0	8.4	9.3	100

The syrup described in Table A was filtered and diluted to 25.4 g DS/100 g solution with ion-exchanged water and was fed to a column containing a crosslinked strong acid cation exchange resin in the calcium form. The physical parameters of the column appear in Table B. Diluted syrup (169 g) was then fed to the column, which was maintained at 65° C., after which the column was eluted using water at a flow rate of 50 mL/min.

TABLE B

Physical Parameters of the Column	
Resin Type	FINEX CS11GC
Ion form	Ca <sup>2+</sup>
Crosslinking, % divinyl benzene	5.5

46

TABLE B-continued

Physical Parameters of the Column	
Particle size (mm)	0.34
Bed length (m)	1.69
Column diameter (m)	0.093

Two fractions containing fructose were isolated. Fraction 6.1 eluted between 73 and 103 minutes, and fraction 6.2 eluted between 103 and 120 minutes. Of the fructose fed to the chromatographic separation, 93.0% of the fructose fed to the column was isolated in fraction 6.2 in >90% purity. The product distribution in each fraction (6.1 and 6.2) as measured by HPLC appears in Table C.

TABLE C

Product Distribution of Chromatographic Fractions Containing Fructose from a Hydrolyzed Glucan Filtrate								
Frac- tion	Su- crose	Leucrose	Glu- cose	Fruc- tose	DP3+	Others	Total	% Fruc- tose recov- ered
6.1	7.7	13.9	63.9	7.3	1.5	5.7	100	5.9
6.2	0.0	0.6	3.0	91.8	0.0	4.6	100	93.0

The reduced separation efficiency in this example compared to Example 5 can be attributed to differences in the scale of the column and the higher glucose fraction of the sample. Even so, chromatographic purification of this material resulted in an increased yield of high purity fructose syrup compared to that achieved in Example 5, in which syrup was chromatographically prepared from a glucan filtrate that had not been hydrolyzed by a transglucosidase. As the feed composition for this separation comprised 47% fructose (Table A), 43.7% of the total stream was recovered as a fructose syrup with >90 wt % DS fructose. This 43.7% recovery is significantly better than the 34.5% recovery in Example 5.

Thus, fructose isolation from a glucan filtrate that has been hydrolyzed with transglucosidase results in an increased yield of fructose compared to when isolating fructose from a non-hydrolyzed glucan filtrate.

#### Example 7

##### Production of Ethanol by Fermenting a Filtrate of a Glucan Synthesis Reaction

This example discloses yeast fermentation of glucan filtrate to ethanol.

Yeast (*S. cerevisiae*) cream (Tonon mill, Brazil) was washed by suspending the cream in tap water (2.4 L, optical density of 65 at 600 nm) and then centrifuging the yeast cream for 5 minutes using a LEGEND XTR centrifuge (Thermo Scientific) at 4500 g. After decanting the supernatant, the yeast cells were resuspended and concentrated by centrifugation two additional times. After the third wash, the pH was adjusted to 2 by addition of 5 wt % sulfuric acid. The optical density was measured using a GENESYS 20 4001 spectrophotometer (Thermo Scientific) and adjusted to 100 at 600 nm by addition of tap water. The adjusted yeast cream (1.5 L) was added to a 7.5-L BIOFLO310 fermenter vessel (New Brunswick). The fermenter was set to maintain temperature at 30° C. and agitation at 100 rpm. Although pH

47

was measured during fermentation, it was not controlled by the addition of acid or base solutions.

A feed solution containing yeast extract (10 g/L), peptone (20 g/L), and 200 g/L of sugars from a glucan filtrate was prepared and sterilized using a PHOENIX AV-250 PLUS autoclave at 121° C. for 15 minutes. The feed solution was allowed to cool to 25° C. (room temperature) before the fermentation began. The sterilized feed solution (3.5 L) was added to the fermenter over approximately 5 hours at a rate of 684 mL/hr, and the fermentation was allowed to proceed for 22 hours.

Periodic samples were taken during the fermentation and analyzed for optical density using a GENESYS 20 4001 spectrophotometer, Brix using a PAL-3 refractometer (Atago), and sugar and ethanol concentrations by HPLC (General Methods). These results are summarized in Table 10.

TABLE 10

Feed and Time Course Fermentation Profiles for the First Ethanol Fermentation								
Time (hr)	Sucrose	Leucrose	Glucose	Fru- tose	DP2	DP3+	Total Sugar	EtOH
Feed	9	70	19	76	7	19	200	0
0	0.2	0.0	0.0	0.1	—	—	—	7
1	<1	18	0.3	<1	—	—	—	15
2	<1	30	0.4	<1	—	—	—	21
3	<1	40	0.0	<1	—	—	—	25
4	<1	46	0.0	<1	—	—	—	28
5	<1	49	0.0	<1	—	—	—	29
6	<1	53	0.0	<1	—	—	—	32
8	<1	53	0.0	<1	—	—	—	33
22	<1	53	0.0	<1	5	18	76	33

Concentrations (g/L) of ethanol (EtOH) and sugar compounds in the feed and at various fermentation time points (0-22 hours) are listed.

When the fermentation was over, the yeast cells were separated by centrifugation using a LEGEND XTR centrifuge at 4500 g for 5 minutes. After decanting the supernatant, the yeast were resuspended and concentrated by centrifugation two additional times. After the third wash, the pH was adjusted to 2 by addition of 5 wt % sulfuric acid. The optical density was measured using a GENESYS 20 4001 spectrophotometer and adjusted to 100 at 600 nm by addition of tap water. Two additional fermentation cycles, each using fresh feed, were performed using recycled yeast cells from the previous fermentation following the same conditions described above. The fermentation results obtained using first-time and second-time recycled yeast are provided in Tables 11 and 12, respectively.

TABLE 11

Feed and Time Course Fermentation Profiles Using the First Recycle of Yeast Cells								
Time (hr)	Sucrose	Leucrose	Glucose	Fru- tose	DP2	DP3+	Total Sugar	EtOH
Feed	13	69	21	77	7	18	206	0
0	0	4	0	0	—	—	—	5
1	<1	19	0	<1	—	—	—	18
4	<1	35	0	<1	—	—	—	23
4	<1	40	0	<1	—	—	—	26
5	<1	45	0	<1	—	—	—	29
6	<1	53	0	<1	—	—	—	32
7	<1	50	0	<1	—	—	—	32
7	<1	51	0	<1	—	—	—	33
21	<1	42	0	<1	6	18	65	37

Concentrations (g/L) of ethanol (EtOH) and sugar compounds in the feed and at various fermentation time points (0-21 hours) are listed.

48

TABLE 12

Feed and Time Course Fermentation Profiles Using the Second Recycle of Yeast Cells								
Time (hr)	Sucrose	Leucrose	Glucose	Fru- tose	DP2	DP3+	Total Sugar	EtOH
Feed	10	70	19	76	6	19	201	0
0	<1	0	0	<1	—	—	—	11
1	<1	32	0	<1	—	—	—	24
4	<1	40	0	<1	—	—	—	29
4	<1	45	0	<1	—	—	—	31
5	<1	46	0	<1	—	—	—	33
6	<1	45	0	<1	—	—	—	34
6	<1	16	0	<1	—	—	—	48
7	<1	7	0	<1	—	—	—	52
21	<1	5	0	<1	5	16	27	54

Concentrations (g/L) of ethanol (EtOH) and sugar compounds in the feed and at various fermentation time points (0-21 hours) are listed.

Very little leucrose was consumed in the first fermentation, although the yeast cells started to acclimate and consume leucrose by the second recycle. Ethanol fermentation titers increased from 33 g/L (Table 10, 22 hours) to 54 g/L (Table 12, 21 hours) after three fermentation cycles with recycled yeast, although significant amounts of leucrose were present in the medium, even after the last cycle.

Thus, glucan filtrate can be used in a fermentation process to produce ethanol.

## Example 8

Production of Ethanol by Fermenting Hydrolyzed  
Glucan Filtrate

This example demonstrates that fermenting a glucan filtrate in which the leucrose and oligosaccharide byproduct components have previously been saccharified results in increased ethanol yields.

Fermentations were performed following the procedure outlined in Example 7, but using a glucan filtrate that was previously treated with a transglucosidase (TG L-2000, SEQ ID NO:1). Hydrolyzed glucan filtrate was prepared as follows. Glucan filtrate was adjusted to 300 g sugars/L and then the pH was adjusted to 4.0 using 1.0 M sodium hydroxide and 5 wt % sulfuric acid. The final volume of this preparation was 6.75 L. The filtrate solution was then sterilized using a PHOENIX AV-250 PLUS autoclave at 121° C. for 15 minutes, and then the temperature was adjusted to 60° C. TG L-2000 enzyme extract as described in Table 4 (135 mL) was mixed with the sterilized filtrate and the solution was incubated in an incubator-shaker (IKA KS4000) at 60° C. and 100 rpm for 72 hours. Hydrolyzed glucan filtrate was thus prepared.

Yeast (*S. cerevisiae*) cream (Bom Retiro mill, Brazil) was washed by suspending the cream in tap water (2.4 L, optical density of 65 at 600 nm) and then centrifuging the yeast cream for 5 minutes using a LEGEND XTR centrifuge at 4500 g. After decanting the supernatant, the yeast were resuspended and concentrated by centrifugation two additional times. After the third wash, the pH was adjusted to 4.5 by addition of 5 wt % sulfuric acid and the optical density was measured using a GENESYS 20 4001 spectrophotometer and adjusted to 100 at 600 nm by addition of tap water. The adjusted yeast cream (1.5 L) was added to a 7.5-L BIOFLO310 fermenter vessel. The fermenter was set to maintain temperature at 30° C., agitation at 100 rpm, and pH at 4.5 using 4 M aqueous ammonium hydroxide or 5 wt % aqueous sulfuric acid.

49

A feed solution containing yeast extract (10 g/L), peptone (20 g/L), and 200 g/L of sugars from the hydrolyzed filtrate was prepared and sterilized using a PHOENIX AV-250 Plus autoclave at 121° C. for 15 minutes. The feed solution was allowed to cool to 25° C. (room temperature) before the fermentation began. The sterilized feed solution (3.5 L) was added to the fermenter over approximately 5 hours at a rate of 684 mL/hr, and the fermentation was allowed to proceed for 22 hours.

Periodic samples were taken during the fermentation and analyzed for optical density using a GENESYS 20 4001 spectrophotometer, Brix using a PAL-3 refractometer, and sugar and ethanol concentrations by HPLC (General Methods). These results are summarized in Table 13.

TABLE 13

Feed and Time Course Fermentation Profiles for the First Ethanol Fermentation Using Hydrolyzed Glucan Filtrate								
Time (hr)	Sucrose	Leucrose	Glucose	Fructose	DP2	DP3+	Total Sugar	EtOH
Feed	7	4	65	97	11	3	186	0
0	0	0	0	0	—	—	—	7
1	<1	1	<1	<1	—	—	—	20
2	<1	3	<1	<1	—	—	—	32
3	<1	4	<1	<1	—	—	—	40
4	<1	4	<1	<1	—	—	—	49
5	<1	4	<1	<1	—	—	—	53
6	<1	5	<1	<1	—	—	—	55
8	<1	5	<1	<1	—	—	—	57
22	<1	5	<1	<1	8	3	16	54

Concentrations (g/L) of ethanol (EtOH) and sugar compounds in the feed and at various fermentation time points (0-22 hours) are listed.

When the fermentation was over, the yeast cells were separated by centrifugation using a LEGEND XTR centrifuge at 4500 g for 5 minutes. After decanting the supernatant, the yeast cells were resuspended and concentrated by centrifugation two additional times. After the third wash, the pH was adjusted to 2 by addition of 5 wt % sulfuric acid. The optical density was measured using a GENESYS 20 4001 spectrophotometer and adjusted to 100 at 600 nm by addition of tap water. Two additional fermentation cycles, each using fresh feed, were performed using recycled yeast cells from the previous fermentation following the same conditions described above. The fermentation results obtained using first-time and second-time recycled yeast cells are provided in Tables 14 and 15, respectively.

TABLE 14

Feed and Time Course Fermentation Profiles Using the First Recycle of Yeast Cells with Hydrolyzed Glucan Filtrate								
Time (hr)	Sucrose	Leucrose	Glucose	Fructose	DP2	DP3+	Total Sugar	EtOH
Feed	7	4	69	104	7	4	194	0
0	<1	0	<1	<1	—	—	—	10
1	<1	7	<1	<1	—	—	—	25
4	<1	5	<1	<1	—	—	—	39
4	<1	4	<1	<1	—	—	—	45
5	<1	5	<1	<1	—	—	—	51
6	<1	5	<1	<1	—	—	—	57
6,2	<1	5	<1	<1	—	—	—	60
7	<1	5	<1	<1	—	—	—	59
21	<1	5	<1	<1	9	5	19	58

Concentrations (g/L) of ethanol (EtOH) and sugar compounds in the feed and at various fermentation time points (0-21 hours) are listed.

50

TABLE 15

Feed and Time Course Fermentation Profiles Using the Second Recycle of Yeast Cells with Hydrolyzed Glucan Filtrate								
Time (hr)	Sucrose	Leucrose	Glucose	Fructose	DP2	DP3+	Total Sugar	EtOH
Feed	7	4	70	105	7	5	197	0
0	0	0	0	0	—	—	—	10
1	<1	7	<1	<1	—	—	—	25
3,5	<1	5	<1	<1	—	—	—	39
4	<1	4	<1	<1	—	—	—	45
5	<1	5	<1	<1	—	—	—	51
6	<1	5	<1	<1	—	—	—	57
6,2	<1	5	<1	<1	—	—	—	60
7	<1	5	<1	<1	—	—	—	59
21	<1	5	<1	<1	9	5	19	58

Concentrations (g/L) of ethanol (EtOH) and sugar compounds in the feed and at various fermentation time points (0-21 hours) are listed.

All of the fermentations were essentially complete within about six hours of initiating fermentation, and resulted in ethanol titers of 57-60.0 g/L. Comparing these fermentations with those in Example 7 demonstrates that hydrolyzing a glucan filtrate before subjecting it to fermentation results in faster and greater ethanol yields than those obtained from fermentations using non-hydrolyzed glucan filtrate.

Thus, fermenting a glucan filtrate in which the leucrose and oligosaccharide byproduct components have been saccharified results in increased ethanol yields at faster rates. This saccharification can be done using a transglucosidase, for example.

## Example 9

## Simultaneous Saccharification and Fermentation of a Glucan Filtrate Solution

This example discloses that simultaneous saccharification and fermentation of a feed containing glucan filtrate can result in enhanced fermentation properties.

Yeast (*S. cerevisiae*) cream (Bom Retiro mill, Brazil) was washed by suspending the cream in tap water (2.4 L, optical density of 65 at 600 nm) and then centrifuging the yeast cream for 5 minutes using a LEGEND XTR centrifuge at 4500 g. After decanting the supernatant, the yeast cells were resuspended and concentrated by centrifugation two additional times. After the third wash, the pH was adjusted to 4.5 by addition of 5 wt % sulfuric acid and the optical density was measured using a GENESYS 20 4001 spectrophotometer and adjusted to 100 at 600 nm by addition of tap water. The adjusted yeast cream (1.5 L) was added to a 7.5-L BIOFLO310 fermenter vessel. The fermenter was set to maintain temperature at 30° C., agitation at 100 rpm, and pH at 4.5 using 4 M aqueous ammonium hydroxide or 5 wt % aqueous sulfuric acid.

A feed solution containing yeast extract (10 g/L), peptone (20 g/L), and 200 g/L of sugars from a glucan filtrate was prepared and sterilized using a PHOENIX AV-250 PLUS autoclave at 121° C. for 15 minutes. The feed solution was allowed to cool to 25° C. (room temperature) before the fermentation began. TG L-2000 transglucosidase enzyme extract as described in Table 4 (1% v/v) was added to the sterilized feed solution immediately before adding the solution to the fermenter. The feed solution (3.5 L) containing TG L-2000 enzyme was added to the fermenter over approximately 5 hours at 684 mL/hr, and the fermentation was allowed to proceed for 48 hours.

Periodic samples were taken during the fermentation and analyzed for optical density using a GENESYS 20 4001 spectrophotometer, Brix using a PAL-3 refractometer (Atago), and sugar and ethanol concentrations by HPLC (General Methods). These results are summarized in Table 16.

TABLE 16

Feed and Time Course Fermentation Profiles for Simultaneous Saccharification and Ethanol Fermentation of Glucan Filtrate								
Time (hr)	Sucrose	Leucrose	Glucose	Fructose	DP2	DP3+	Total Sugar	EtOH
Feed	7	82	12	79	6	20	206	0
0	0	0	0	0	0	0	0	2
1	<1	13	<1	3	—	—	—	11
2	<1	21	<1	4	—	—	—	30
3	<1	21	<1	3	—	—	—	38
4	<1	20	<1	3	11	16	50	43
5	<1	14	<1	2	—	—	—	45
6	<1	<1	<1	2	—	—	—	59
22	<1	<1	<1	1	—	—	—	62
25	<1	<1	<1	<1	—	—	—	63
27	<1	<1	<1	<1	—	—	—	63
31	<1	<1	<1	<1	—	—	—	57
46	<1	<1	<1	<1	—	—	—	57
48	<1	<1	<1	<1	1	11	12	62

Concentrations (g/L) of ethanol (EtOH) and sugar compounds in the feed and at various fermentation time points (0-48 hours) are listed.

The fermentation was nominally complete in 6 hours, similar to the fermentations where the filtrate was hydrolyzed prior to the fermentation step (Example 8), and gave a slightly superior titer of ethanol (62 g/L) compared to using unhydrolyzed filtrate (Example 7). In addition, almost all of the leucrose was consumed by 6 hours (compare Table 16 with Tables 13-15). In addition to adding a saccharifying enzyme, such as TG L-2000, to a feed containing glucan filtrate just prior to fermentation, similar results should be obtained if the saccharifying enzyme is added to the fermentation directly.

Thus, simultaneous saccharification and fermentation of a feed containing glucan filtrate can result in enhanced fermentation properties such as increased (i) consumption of glucan filtrate components (e.g., leucrose) and (ii) ethanol yield and rate of production.

#### Example 10

##### Preparation of Various Alpha-Glucosidases

This example discloses preparing various alpha-glucosidases in addition to those alpha-glucosidases (transglucosidase, glucoamylase, DIAZYME RDF ULTRA) used in some of the foregoing Examples. These additional alpha-glucosidases were tested for hydrolytic activity against oligosaccharides comprising alpha-1,5 glucosyl-fructose linkages or alpha-1,3 and/or alpha-1,6 glucosyl-glucose linkages in Examples 11, 12, 15 and 16 provided below. Discovery of an *Aspergillus clavatus* Alpha-Glucosidase (Acglu1)

A strain of *Aspergillus clavatus* was selected as a potential source of other enzymes that may be useful in various industrial applications. One of the genes identified in *Aspergillus clavatus* encodes an alpha-glucosidase and the sequence of this gene, called "Acglu1", is provided in SEQ ID NO:4. The corresponding protein encoded by SEQ ID NO:4 is provided in SEQ ID NO:5. Acglu1 belongs to Glycosyl hydrolase family 31 based on a PFAM search

(pfam.sanger.ac.uk web link). At the N-terminus, the protein (SEQ ID NO:5) has a signal peptide with a length of 19 amino acids as predicted by SignalP version 4.0 (Nordahl Petersen et al., 2011, *Nature Methods*, 8:785-786). The presence of a signal sequence suggests that Acglu1 is a secreted enzyme. The amino acid sequence of the predicted mature form of Acglu1 is set forth as SEQ ID NO:6.

Expression of *Aspergillus clavatus* Alpha-Glucosidase Acglu1

A synthetic Acglu1 gene was cloned into pTrex3gM expression vector (described in U.S. Patent Appl. Publ. No. 2011/0136197, incorporated herein by reference) and the resulting plasmid was designated as pJG294. The sequence of the Acglu1 gene was confirmed by DNA sequencing.

Plasmid pJG294 was transformed into a quad deleted *Trichoderma reesei* strain (described in WO05/001036) using a biolistic method (Te'o V S et al., *J Microbiol Methods*, 51:393-9, 2002). The protein, which was predicted to comprise SEQ ID NO:6, was secreted into the extracellular medium and filtered culture medium was used to perform SDS-PAGE and alpha-glucosidase activity assays to confirm enzyme expression.

Discovery of *Neosartorya Fischeri* Alpha-glucosidase Nfiglu1

A strain of *Neosartorya fischeri* was selected as a potential source of other enzymes that may be useful in various industrial applications. One of the genes identified in *Neosartorya fischeri* encodes an alpha-glucosidase and the sequence of this gene, called "Nfiglu1", is provided in SEQ ID NO:7. The corresponding protein encoded by SEQ ID NO:7 is provided in SEQ ID NO:8. Nfiglu1 belongs to Glycosyl hydrolase family 31 based on a PFAM search (pfam.sanger.ac.uk web link). At the N-terminus, the protein (SEQ ID NO:8) has a signal peptide with a length of 19 amino acids as predicted by SignalP version 4.0 (Nordahl Petersen et al., 2011, *Nature Methods*, 8:785-786). The presence of a signal sequence suggests that Nfiglu1 is a secreted enzyme. The amino acid sequence of the predicted mature form of Nfiglu1 is set forth as SEQ ID NO: 9.

Expression of *Neosartorya fischeri* Alpha-glucosidase Nfiglu1

A synthetic Nfiglu1 gene was cloned into pTrex3gM expression vector (described in U.S. Patent Appl. Publ. No. 2011/0136197) and the resulting plasmid was designated as pJG295. The sequence of the Nfiglu1 gene was confirmed by DNA sequencing.

Plasmid pJG295 was transformed into a quad deleted *Trichoderma reesei* strain (described in WO05/001036) using a biolistic method (Te'o V S et al., *J Microbiol Methods*, 51:393-9, 2002). The protein, which was predicted to comprise SEQ ID NO:9, was secreted into the extracellular medium and filtered culture medium was used to perform SDS-PAGE and alpha-glucosidase activity assays to confirm enzyme expression.

Discovery of *Neurospora crassa* Alpha-glucosidase Ncrglu1

A strain of *Neurospora crassa* was selected as a potential source of other enzymes that may be useful in various industrial applications. One of the genes identified in *Neurospora crassa* encodes an alpha-glucosidase and the sequence of this gene, called "Ncrglu1", is provided in SEQ ID NO:10. The corresponding protein encoded by SEQ ID NO:10 is provided in SEQ ID NO:11. Ncrglu1 belongs to Glycosyl hydrolase family 31 based on a PFAM search (pfam.sanger.ac.uk web link). At the N-terminus, the protein (SEQ ID NO:11) has a signal peptide with a length of 22 amino acids as predicted by SignalP version 4.0 (Nordahl Petersen et al., 2011, *Nature Methods*, 8:785-786). The

presence of a signal sequence suggests that Ncrglu1 is a secreted enzyme. The amino acid sequence of the predicted mature form of Ncrglu1 is set forth as SEQ ID NO:12.

Expression of *Neurospora crassa* Alpha-Glucosidase Ncrglu1

A synthetic Ncrglu1 gene was cloned into pTrex3gM expression vector (described in U.S. Patent Appl. Publ. No. 2011/0136197) and the resulting plasmid was designated as pJG296. The sequence of the Ncrglu1 gene was confirmed by DNA sequencing.

Plasmid pJG296 was transformed into a quad deleted *Trichoderma reesei* strain (described in WO05/001036) using a biolistic method (Te'o V S et al., J Microbiol Methods, 51:393-399, 2002). The protein, which was predicted to comprise SEQ ID NO:12, was secreted into the extracellular medium and filtered culture medium was used to perform SDS-PAGE and alpha-glucosidase activity assays to confirm enzyme expression.

Discovery of *Rasamsonia composticola* Alpha-glucosidase TauSec098

A strain of *Rasamsonia composticola* was selected as a potential source of other enzymes that may be useful in various industrial applications. One of the genes identified in *Rasamsonia composticola* encodes an alpha-glucosidase and the sequence of this gene, called "TauSec098", is provided in SEQ ID NO:13. The corresponding protein encoded by SEQ ID NO:13 is provided in SEQ ID NO:14. TauSec098 belongs to Glycosyl hydrolase family 31 and contains an N-terminal CBM 20 domain based on a PFAM search (pfam.sanger.ac.uk web link). At the N-terminus, the protein (SEQ ID NO:14) has a signal peptide with a length of 22 amino acids as predicted by SignalP version 4.0 (Nordahl Petersen et al., 2011, *Nature Methods*, 8:785-786). The presence of a signal sequence suggests that TauSec098 is a secreted enzyme. The amino acid sequence of the predicted mature form of TauSec098 is set forth as SEQ ID NO:15.

Expression of *Rasamsonia Composticola* Alpha-glucosidase TauSec098

A synthetic TauSec098 gene was cloned into the *Trichoderma reesei* expression vector pGXT (a pTTT-derived plasmid) by Generay Biotech Co. (Shanghai, China) and the resulting plasmid was designated as pGX256-TauSec098. The sequence of the TauSec098 gene was confirmed by DNA sequencing.

Plasmid pGX256-TauSec098 was transformed into a quad-deleted *Trichoderma reesei* strain (described in WO05/001036) using protoplast transformation (Te'o et al., J. Microbiol. Methods 51:393-399, 2002). Transformants were selected on a medium containing acetamide as a sole source of nitrogen (acetamide 0.6 g/L; cesium chloride 1.68 g/L; glucose 20 g/L; potassium dihydrogen phosphate 15 g/L; magnesium sulfate heptahydrate 0.6 g/L; calcium chloride dihydrate 0.6 g/L; iron (II) sulfate 5 mg/L; zinc sulfate 1.4 mg/L; cobalt (II) chloride 1 mg/L; manganese (II) sulfate 1.6 mg/L; agar 20 g/L; pH 4.25). Transformed colonies (about 50-100) appeared in about 1 week. After growth on acetamide plates, the spores of transformants were collected and transferred into new acetamide agar plates. After 5 days of growth on acetamide plates,  $1 \times 10^8$  spores were inoculated into 30 ml Glucose/Sophorose defined media in a 250-mL shake flask. The shake flask was shook at 28° C. for 5 days. Supernatants from these cultures were used to confirm expression (SDS PAGE) and activity of mature TauSec098 enzyme (SEQ ID NO:15).

Discovery of *Rasamsonia composticola* Alpha-glucosidase TauSec099

A strain of *Rasamsonia composticola* was selected as a potential source of other enzymes that may be useful in various industrial applications. One of the genes identified in *Rasamsonia composticola* encodes an alpha-glucosidase and the sequence of this gene, called "TauSec099", is provided in SEQ ID NO:16. The corresponding protein encoded by SEQ ID NO:16 is provided in SEQ ID NO:17. TauSec099 belongs to Glycosyl hydrolase family 31 based on a PFAM search (pfam.sanger.ac.uk web link). At the N-terminus, the protein (SEQ ID NO:17) has a signal peptide with a length of 17 amino acids as predicted by SignalP version 4.0 (Nordahl Petersen et al., 2011, *Nature Methods*, 8:785-786). The presence of a signal sequence suggests that TauSec099 is a secreted enzyme. The amino acid sequence of the predicted mature form of TauSec099 is set forth as SEQ ID NO:18.

Expression of *Rasamsonia composticola* Alpha-glucosidase TauSec099

A synthetic TauSec099 gene was cloned into the *Trichoderma reesei* expression vector pGXT (a pTTT-derived plasmid) by Generay Biotech Co. (Shanghai, China) and the resulting plasmid was designated as pGX256-TauSec099. The sequence of the TauSec099 gene was confirmed by DNA sequencing.

Plasmid pGX256-TauSec099 was transformed into a quad-deleted *Trichoderma reesei* strain (described in WO05/001036) using protoplast transformation (Te'o et al., J. Microbiol. Methods 51:393-399, 2002). Transformants were selected on a medium containing acetamide as a sole source of nitrogen (acetamide 0.6 g/L; cesium chloride 1.68 g/L; glucose 20 g/L; potassium dihydrogen phosphate 15 g/L; magnesium sulfate heptahydrate 0.6 g/L; calcium chloride dihydrate 0.6 g/L; iron (II) sulfate 5 mg/L; zinc sulfate 1.4 mg/L; cobalt (II) chloride 1 mg/L; manganese (II) sulfate 1.6 mg/L; agar 20 g/L; pH 4.25). Transformed colonies (about 50-100) appeared in about 1 week. After growth on acetamide plates, the spores of transformants were collected and transferred into new acetamide agar plates. After 5 days of growth on acetamide plates,  $1 \times 10^8$  spores were inoculated into 30 ml Glucose/Sophorose defined media in a 250-mL shake flask. The shake flask was shook at 28° C. for 5 days.

Supernatants from these cultures were used to confirm expression (SDS PAGE) and activity of mature TauSec099 enzyme (SEQ ID NO:18).

Sequences of *Bifidobacterium longum* Alpha-glucosidase BloGlu1

An alpha-glucosidase gene, "BloGlu1", was identified from *Bifidobacterium longum* subsp. *longum* JDM301. The nucleic acid sequence for the BloGlu1 gene (SEQ ID NO:19, GENBANK Acc. No. NC014169.1, complement sequence from positions 140600 to 142414) and the amino acid sequence of the hypothetical protein (SEQ ID NO:20) encoded by SEQ ID NO:19 were found in GENBANK Acc. No. YP\_003660432.1.

Expression of *Bifidobacterium longum* Alpha-glucosidase BloGlu1

The DNA sequence encoding the entire BloGlu1 protein (SEQ ID NO:20) was optimized for expression in *B. subtilis*, then synthesized (yielding SEQ ID NO:21) and inserted into the p3JM plasmid by Generay Biotech Co. (Shanghai, China), resulting in p3JM-BloGlu1. The p3JM-BloGlu1 plasmid contains an aprE promoter to drive expression of the optimized BloGlu1 sequence (SEQ ID NO:21).

Plasmid p3JM-BloGlu1 was used to transform *B. subtilis* cells (degUHy32, ΔnprB, Δvpr, Δepr, ΔscoC, ΔwprA, Δmpr, ΔispA, Δbpr), and the transformed cells were spread on Luria Agar plates supplemented with 5 ppm chloramphenicol. A colony with correct insertion, as confirmed by PCR and sequencing, was selected and subjected to fermentation in a 250-mL shake flask with MBD medium (a MOPS-based defined medium supplemented with an additional 5 mM CaCl<sub>2</sub>) to express BloGlu1 protein (SEQ ID NO:20).

Sequences of *Bifidobacterium longum* Alpha-glucosidase BloGlu2

An alpha-glucosidase, BloGlu2, was identified from *Bifidobacterium longum*. The amino acid sequence (SEQ ID NO:22) of BloGlu2 was found in the NCBI database (GENBANK Acc. No. WP\_007054665.1).

Expression of *Bifidobacterium longum* Alpha-glucosidase BloGlu2

A DNA sequence encoding BloGlu2 protein was optimized for expression in *B. subtilis*, then synthesized (yielding SEQ ID NO:23) and inserted into the p3JM plasmid by Genaray Biotech Co., resulting in p3JM-BloGlu2. SEQ ID NO:23 encodes the amino acid sequence of SEQ ID NO:24. The p3JM-BloGlu2 plasmid contains an aprE promoter to drive expression of the optimized BloGlu2 sequence (SEQ ID NO:23).

Plasmid p3JM-BloGlu2 was used to transform *B. subtilis* cells (degUHy32, ΔnprB, Δvpr, Δepr, ΔscoC, ΔwprA, Δmpr, ΔispA, Δbpr), and the transformed cells were spread on Luria Agar plates supplemented with 5 ppm chloramphenicol. A colony with correct insertion, as confirmed by PCR and sequencing, was selected and subjected to fermentation in a 250-mL shake flask with MBD medium (a MOPS-based defined medium supplemented with an additional 5 mM CaCl<sub>2</sub>) to express BloGlu2 protein (SEQ ID NO:24).

Sequences of *Bifidobacterium longum* Alpha-glucosidase BloGlu3

An alpha-glucosidase gene, "BloGlu3", was identified from *Bifidobacterium longum* subsp. *longum* F8. The nucleic acid sequence for the BloGlu3 gene (SEQ ID NO:25, GENBANK Acc. No. NC\_021008.1, positions 2130627 to 2132441), and the amino acid sequence of the hypothetical protein (SEQ ID NO:26) encoded by SEQ ID NO:25 were found in GENBANK Acc. No. YP\_007768249.1.

Expression of *Bifidobacterium longum* Alpha-glucosidase BloGlu3

The DNA sequence encoding the entire BloGlu3 protein (SEQ ID NO:26) was optimized for expression in *B. subtilis*, then synthesized (yielding SEQ ID NO:27) and inserted into the p3JM plasmid by Genaray Biotech Co., resulting in p3JM-BloGlu3. The p3JM-BloGlu3 plasmid contains an aprE promoter to drive expression of the optimized BloGlu3 sequence (SEQ ID NO:27).

Plasmid p3JM-BloGlu3 was used to transform *B. subtilis* cells (degUHy32, ΔnprB, Δvpr, Δepr, ΔscoC, ΔwprA, Δmpr, ΔispA, Δbpr), and the transformed cells were spread on Luria Agar plates supplemented with 5 ppm chloramphenicol. A colony with correct insertion, as confirmed by PCR and sequencing, was selected and subjected to fermentation in a 250-mL shake flask with MBD medium (a MOPS-based defined medium supplemented with an additional 5 mM CaCl<sub>2</sub>) to express BloGlu3 protein (SEQ ID NO:26).

Sequences of *Bifidobacterium pseudolongum* Alpha-glucosidase BpsGlu1

An alpha-glucosidase, BpsGlu1, was identified from *Bifidobacterium pseudolongum*. The amino acid sequence (SEQ ID NO:28) of BpsGlu1 was found in the NCBI database (GENBANK Acc. No. WP\_022858408.1).

Expression of *Bifidobacterium pseudolongum* Alpha-glucosidase BpsGlu1

A DNA sequence encoding BpsGlu1 protein was optimized for expression in *B. subtilis*, then synthesized (yielding SEQ ID NO:29) and inserted into the p3JM plasmid by Genaray Biotech Co., resulting in p3JM-BpsGlu1. SEQ ID NO:29 encodes the amino acid sequence of SEQ ID NO:30. The p3JM-BpsGlu1 plasmid contains an aprE promoter to drive expression of the optimized BpsGlu1 sequence (SEQ ID NO:29).

Plasmid p3JM-BpsGlu1 was used to transform *B. subtilis* cells (degUHy32, ΔnprB, Δvpr, Δepr, ΔscoC, ΔwprA, Δmpr, ΔispA, Δbpr), and the transformed cells were spread on Luria Agar plates supplemented with 5 ppm chloramphenicol. A colony with correct insertion, as confirmed by PCR and sequencing, was selected and subjected to fermentation in a 250-mL shake flask with MBD medium (a MOPS-based defined medium supplemented with an additional 5 mM CaCl<sub>2</sub>) to express BpsGlu1 protein (SEQ ID NO:30).

Sequences of *Bifidobacterium thermophilum* Alpha-glucosidase BthGlu1

An alpha-glucosidase gene, "BthGlu1", was identified from *Bifidobacterium thermophilum* RBL67. The nucleic acid sequence of the BthGlu1 gene (SEQ ID NO:31, GENBANK Acc. No. NC\_020546.1, positions 150690 to 152495), and the amino acid sequence of the hypothetical protein (SEQ ID NO:32) encoded by SEQ ID NO:31 were found in GENBANK Acc. No. YP\_007592840.1.

Expression of *Bifidobacterium thermophilum* Alpha-glucosidase BthGlu1

The DNA sequence encoding the entire BthGlu1 protein (SEQ ID NO:32) was optimized for expression in *B. subtilis*, then synthesized (yielding SEQ ID NO:33) and inserted into the p3JM plasmid by Genaray Biotech Co., resulting in p3JM-BthGlu1. The p3JM-BthGlu1 plasmid contains an aprE promoter to drive expression of the optimized BthGlu1 sequence (SEQ ID NO:33).

Plasmid p3JM-BthGlu1 was used to transform *B. subtilis* cells (degUHy32, ΔnprB, Δvpr, Δepr, ΔscoC, ΔwprA, Δmpr, ΔispA, Δbpr), and the transformed cells were spread on Luria Agar plates supplemented with 5 ppm chloramphenicol. A colony with correct insertion, as confirmed by PCR and sequencing, was selected and subjected to fermentation in a 250-mL shake flask with MBD medium (a MOPS-based defined medium supplemented with an additional 5 mM CaCl<sub>2</sub>) to express BthGlu1 protein (SEQ ID NO:32).

Sequences of *Bifidobacterium breve* Alpha-glucosidase BbrGlu2

An alpha-glucosidase, BbrGlu2, was identified from *Bifidobacterium breve*. The amino acid sequence (SEQ ID NO:34) of BbrGlu2 was found in the NCBI database (GENBANK Acc. No. WP\_003827971.1).

Expression of *Bifidobacterium breve* Alpha-glucosidase BbrGlu2

A DNA sequence encoding BbrGlu2 protein was optimized for expression in *B. subtilis*, then synthesized (yielding SEQ ID NO:35) and inserted into the p3JM plasmid by Genaray Biotech Co., resulting in p3JM-BbrGlu2. SEQ ID NO:35 encodes the amino acid sequence of SEQ ID NO:36. The p3JM-BbrGlu2 plasmid contains an aprE promoter to drive expression of the optimized BbrGlu2 sequence (SEQ ID NO:35).

Plasmid p3JM-BbrGlu2 was used to transform *B. subtilis* cells (degUHy32, ΔnprB, Δvpr, Δepr, ΔscoC, ΔwprA, Δmpr, ΔispA, Δbpr), and the transformed cells were spread on Luria Agar plates supplemented with 5 ppm chloramphenicol. A colony with correct insertion, as confirmed by PCR

57

and sequencing, was selected and subjected to fermentation in a 250-mL shake flask with MBD medium (a MOPS-based defined medium supplemented with an additional 5 mM CaCl<sub>2</sub>) to express SEQ ID NO:36.

Sequences of *Bifidobacterium breve* Alpha-glucosidase BbrGlu5

An alpha-glucosidase gene, "BbrGlu5", was identified from *Bifidobacterium breve* ACS-071-V-Sch8b. The nucleic acid sequence of the BbrGlu5 gene (SEQ ID NO:37, GENBANK Acc. No. NC\_017218.1, complement of sequence from positions 2241075 to 2242895), and the amino acid sequence of the hypothetical protein (SEQ ID NO:38) encoded by SEQ ID NO:37 were found in GENBANK Acc. No. YP\_005583701.1.

Expression of *Bifidobacterium breve* Alpha-glucosidase BbrGlu5

The DNA sequence encoding the entire BbrGlu5 protein (SEQ ID NO:38) was optimized for expression in *B. subtilis*, then synthesized (yielding SEQ ID NO:39) and inserted into the p3JM plasmid by Genaray Biotech Co., resulting in p3JM-BbrGlu5. The p3JM-BbrGlu5 plasmid contains an aprE promoter to drive expression of the optimized BbrGlu5 sequence (SEQ ID NO:39).

Plasmid p3JM-BbrGlu5 was used to transform *B. subtilis* cells (degUHy32, ΔnprB, Δvpr, Δepr, ΔscoC, ΔwprA, Δmpr, ΔispA, Δbpr), and the transformed cells were spread on Luria Agar plates supplemented with 5 ppm chloramphenicol. A colony with correct insertion, as confirmed by PCR and sequencing, was selected and subjected to fermentation in a 250-mL shake flask with MBD medium (a MOPS-based defined medium supplemented with an additional 5 mM CaCl<sub>2</sub>) to express BbrGlu5 protein (SEQ ID NO:38).

Purification of Alpha-glucosidases from Expression Cultures

AclGlu1 and NcrGlu1

Both AclGlu1 (SEQ ID NO:6) and NcrGlu1 (SEQ ID NO:12) alpha-glucosidases were purified using two chromatography steps. For each purification, the crude broth from the shake flask was concentrated, after which ammonium sulfate was added to a final concentration of 2 M. The solution was loaded onto a 50-mL phenyl HP column pre-equilibrated with 20 mM Tris pH 8.0, 2 M ammonium sulfate. The target protein (SEQ ID NO:6 or SEQ ID NO:12) was eluted from the column with 1 M ammonium sulfate, 20 mM Tris pH 8.0. Respective fractions were pooled, concentrated and buffer-exchanged into 20 mM Tris pH 8.0 (buffer A), using a VIVAFLOW 200 ultrafiltration device (Sartorius Stedim). The resulting solution was applied to a 40-mL Q HP column pre-equilibrated with buffer A. The target protein was eluted from the column with 0.3 M NaCl in buffer A. The fractions containing target protein were then pooled and concentrated using 10K AMICON ULTRA-15 devices, and stored in 40% glycerol at -20° C. until usage.

NfiGlu1

NfiGlu1 alpha-glucosidase (SEQ ID NO:9) was purified using two hydrophobic interaction chromatography steps. The crude broth from the shake flask was concentrated, after which ammonium sulfate was added to a final concentration of 1 M. The solution was loaded onto a 50-mL phenyl HP column pre-equilibrated with 20 mM Tris pH 8.0, 1 M ammonium sulfate. The target protein (SEQ ID NO:9) flowed through the column. Flow-through fractions were pooled, after which ammonium sulfate was added to a final concentration of 2 M. The solution was loaded onto the same phenyl HP column pre-equilibrated with 20 mM Tris pH 8.0, 2 M ammonium sulfate. The target protein was eluted from the column with 1 M ammonium sulfate, 20 mM Tris pH 8.0.

58

The fractions containing target protein were then pooled and concentrated using 10K AMICON ULTRA-15 devices, and stored in 40% glycerol at -20° C. until usage.

TauSec098 and TauSec099

Both TauSec098 (SEQ ID NO:15) and TauSec099 (SEQ ID NO:18) alpha-glucosidases were purified via hydrophobic interaction chromatography. For each purification, ammonium sulphate was added to about 180 mL of concentrated crude broth from a 7-L fermenter to a final concentration of 1 M. This solution was then loaded onto a 50-mL HIPREP phenyl-FF Sepharose column (GE Healthcare) pre-equilibrated with 20 mM sodium acetate pH 5.0.1 M ammonium sulphate (buffer A). After washing with the same buffer with three column volumes (CVs), the column was eluted stepwise with 75%, 50% and 0% buffer A using three CVs each, followed by two CVs of MILLIQ H<sub>2</sub>O. All fractions were analyzed by SDS-PAGE. The target protein (SEQ ID NO:15 or SEQ ID NO:18) was mainly present in the flow-through fraction, which was concentrated and buffer-exchanged to remove excess ammonium sulfate using 10 KDa AMICON ULTRA-15 devices. The final product, which was greater than 90% pure, was stored in 40% glycerol at -80° C. until usage.

BloGlu1, BloGlu2 and BloGlu3

BloGlu1 (SEQ ID NO:20), BloGlu2 (SEQ ID NO:24) and BloGlu3 (SEQ ID NO:26) alpha-glucosidases were all purified in three steps. For each purification, the crude broth from a 1-L DASGIP fermenter was concentrated, after which ammonium sulfate was added to 60% saturation. The solution was stirred at 4° C. for 1 hr, and then centrifuged at 8000×g for 30 min. The resulting pellet was re-suspended in 20 mM Tris pH 8.0 (buffer A). Ammonium sulfate was added to the resulting solution to a final concentration of 1 M; this preparation was then loaded onto a 40-mL HiPrep™ Phenyl FF column pre-equilibrated with 20 mM Tris pH 8.0, 1 M ammonium sulfate (buffer B). After washing, the column was eluted stepwise with 75%, 50%, and 0% buffer B and H<sub>2</sub>O in three column volumes each. All fractions were analyzed using SDS-PAGE and activity assays. The fractions containing target protein (SEQ ID NO:20, SEQ ID NO:24, or SEQ ID NO:26) were pooled, concentrated and subsequently loaded onto a HiLoad™ 26/60 Superdex™ 75 column pre-equilibrated with 20 mM sodium phosphate pH 7.0, 0.15 M NaCl. Flow-through fractions containing the target protein were then pooled and concentrated using 10K AMICON ULTRA-15 devices, and stored in 40% glycerol at -20° C. until usage.

BpsGlu1 and BthGlu1

Both BpsGlu1 (SEQ ID NO:30) and BthGlu1 (SEQ ID NO:32) alpha-glucosidases were purified in two steps. For each purification, the crude broth from a 1-L DASGIP fermenter was concentrated, after which ammonium sulfate was added to 60% saturation. The solution was stirred at 4° C. for 1 hr, and then centrifuged at 8000×g for 30 min. The resulting pellet was re-suspended in 20 mM Tris pH 8.0 (buffer A). Ammonium sulfate was added to the resulting solution to a final concentration of 1 M; this preparation was then loaded onto a 40-mL HiPrep™ Phenyl FF column pre-equilibrated with 20 mM Tris pH 8.0, 1 M ammonium sulfate (buffer B). After washing, the column was eluted stepwise with 75%, 50%, and 0% buffer B and H<sub>2</sub>O in three column volumes each. All fractions were analyzed using SDS-PAGE and activity assays. The target protein (SEQ ID NO:30 or SEQ ID NO:32) was present in the eluate from the 0% buffer B elution step; this eluate was pooled and concentrated using 10K AMICON ULTRA-15 devices. The

final product, which was greater than 95% pure, was stored in 40% glycerol at  $-20^{\circ}$  C. until usage.  
BbrGlu2 and BbrGlu5

Both BbrGlu2 (SEQ ID NO:36) and BbrGlu5 (SEQ ID NO:38) alpha-glucosidases were purified in four steps. For each purification, the crude broth from a 1-L DASGIP fermenter was concentrated, after which ammonium sulfate was added to 60% saturation. The solution was stirred at  $4^{\circ}$  C. for 1 hr, and then centrifuged at  $8000\times g$  for 30 min. The resulting pellet was re-suspended in 20 mM HEPES pH 7.0 (buffer A). Ammonium sulfate was added to the resulting solution to a final concentration of 1 M; this preparation was then loaded onto a HiPrep™ Phenyl FF column pre-equilibrated with 20 mM HEPES pH 7.0, 1 M ammonium sulfate. The target protein (SEQ ID NO:36 or SEQ ID NO:38) was eluted from the column with 0.5 M ammonium sulfate. Respective fractions were pooled, concentrated and buffer-exchanged into buffer A using a VIVAFLOW 200 ultrafiltration device (Sartorius Stedim). The resulting solution was applied to a HiPrep™ Q FF 16/10 column pre-equilibrated with buffer A. Target protein was eluted from the column with a linear gradient of 0-0.5 M NaCl in buffer A. Fractions containing target protein were pooled, concentrated and subsequently loaded onto a HiLoad™ 26/60 Superdex™ 75 column pre-equilibrated with 20 mM HEPES pH 7.0, 0.15 M NaCl. The fractions containing target protein were then pooled and concentrated using 10K AMICON ULTRA-15 devices, and stored in 40% glycerol at  $-20^{\circ}$  C. until usage.

Thus, various additional alpha-glucosidases were expressed and purified. These alpha-glucosidases were tested for hydrolytic activity against alpha-1,5 glucosyl-fructose linkages and alpha-1,3 and/or alpha-1,6 glucosyl-glucose linkages in Examples 11, 12, 15 and 16 provided below.

#### Example 11

##### Testing Alpha-Glucosidases for Hydrolytic Activity Against Various Glycosidic Linkages

This example discloses testing whether alpha-glucosidases have hydrolytic activity beyond that previously associated with this class of enzymes (EC 3.2.1.20). Alpha-glucosidases from Example 10 were shown to have hydrolytic activity against alpha-1,5 glucosyl-fructose linkages and alpha-1,3 and alpha-1,6 glucosyl-glucose linkages. Substrate Specificity of Alpha-Glucosidases

The substrate specificity of each alpha-glucosidase disclosed in Example was assayed based on the release of glucose from a particular substrate (isomaltose, maltose, panose, leucrose, or nigerose) when the substrate was incubated with alpha-glucosidase. The rate of glucose release was measured using a coupled glucose oxidase/peroxidase (GOX/HRP) method (1980, *Anal. Biochem.* 105:389-397). Glucose release was quantified as the rate of oxidation of 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) by peroxide that was generated from coupled GOX/HRP enzymes reacted with glucose.

Individual substrate solutions were prepared by mixing a 9 mL solution of substrate (1% in water, w/v) with 1 mL of 0.5 M pH 5.0 sodium acetate buffer and 40  $\mu$ L of 0.5 M calcium chloride in a 15-mL conical tube. Coupled enzyme (GOX/HRP) solution with ABTS was prepared in 50 mM sodium acetate buffer (pH 5.0), with the final concentrations of 2.74 mg/mL ABTS, 0.1 U/mL HRP, and 1 U/mL GOX. Serial dilutions of individual alpha-glucosidase samples and

glucose standard were prepared in MILLIQ water. For nigerose, alpha-glucosidase samples were tested with only one dosage at 10 ppm due to a limited stock of substrate solutions. Each alpha-glucosidase sample (10  $\mu$ L) was transferred into a new microtiter plate (Corning 3641) containing 90  $\mu$ L of substrate solution pre-incubated at  $50^{\circ}$  C. for 5 min at 600 rpm. Reactions were carried out at  $50^{\circ}$  C. for 10 min (for isomaltose, maltose, panose, and nigerose substrates), or for 60 min (for leucrose substrate) with shaking (600 rpm) in a THERMOMIXER (Eppendorf). 10  $\mu$ L of each reaction mix, as well as 10  $\mu$ L of serial dilutions of glucose standard, were then quickly transferred to new microtiter plates (Corning 3641), respectively, to which 90  $\mu$ L of ABTS/GOX/HRP solution was then added accordingly. The microtiter plates containing reaction mixes were immediately measured at 405 nm at 11 second intervals for 5 min using a SOFTMAX PRO plate reader (Molecular Devices). The output was the reaction rate,  $V_o$ , for each enzyme concentration. Linear regression was used to determine the slope of the plot  $V_o$  vs. enzyme dose. The specific activity of each alpha-glucosidase was calculated based on the glucose standard curve using Equation 1:

$$\text{Specific Activity (Unit/mg)} = \frac{\text{Slope (enzyme)}}{\text{slope (std)}} \times 1000 \quad (1),$$

where 1 Unit=1  $\mu$ mol glucose/min.

For nigerose, the value of the reaction rate with enzyme dosage at 10 ppm was directly used to indicate enzyme activity.

Using the foregoing method, the specificity of each alpha-glucosidase was determined against each substrate. The activities of an oligo-1,6-glucosidase (purchased from Megazyme, see Table 4) and a transglucosidase (TG L-2000, see Table 4) against each substrate were also measured. The results of this analysis are provided in Table 17.

TABLE 17

Activity of Various Alpha-Glucosidases Against Different Substrates						
SEQ	Enzyme Activity (U/mg) as Measured on:					
Enzyme	ID NO.	Iso-maltose	Maltose	Panose	Leucrose	Nigerose <sup>a</sup>
Oligo-1,6-glucosidase		118.2	0.0	54.3	1.3	19.6
TG L-2000	1	194.0	235.6	127.7	68.9	254.0
AclGlu1	6	255.7	401.9	180.9	113.7	315.1
NfiGlu1	9	521.2	360.0	126.9	89.4	264.3
NerGlu1	12	282.7	34.9	15.9	61.6	200.4
TauSec098	15	54.9	123.8	23.8	1.8	305.6
TauSec099	18	244.0	97.7	50.8	70.6	184.8
BloGlu1	20	71.1	66.9	23.1	2.5	165.0
BloGlu2	24	65.9	86.7	19.9	3.5	217.9
BloGlu3	26	120.1	175.5	31.4	9.0	272.6
BspGlu1	30	64.2	247.9	60.8	27.3	254.6
BthGlu1	32	108.3	93.3	21.1	68.4	128.5
BbrGlu2	38	106.6	167.5	26.9	6.1	258.8
BbrGlu5	38	925.8	0.0	279.7	2.8	22.1

<sup>a</sup>Each enzyme was used at one (10 ppm) against nigerose.

Interestingly, it was found that alpha-glucosidases, besides exhibiting hydrolytic activity against alpha-1,4 glucosyl-glucose linkage (maltose), also exhibit hydrolytic activity against alpha-1,6 glucosyl-glucose linkage (isomaltose), alpha-1,3 glucosyl-glucose linkage (nigerose), and alpha-1,5 glucosyl-fructose linkage (leucrose) (Table 17).

61

Thus, alpha-glucosidases have hydrolytic activity beyond that previously associated with EC 3.2.1.20 enzymes. Specifically, alpha-glucosidases have hydrolytic activity against alpha-1,5 glucosyl-fructose linkages and alpha-1,3 and alpha-1,6 glucosyl-glucose linkages.

Example 12

Hydrolysis of Leucrose and Oligosaccharides in Glucan Reaction Filtrate Using Alpha-Glucosidase

This Example describes using alpha-glucosidase to hydrolyze leucrose and other oligosaccharides present in filtrate obtained from a glucan synthesis reaction. Specifically, the effect of alpha-glucosidases disclosed in Example 10 on the hydrolysis of leucrose and oligosaccharides DP2, DP3 and HS (higher sugars, DP4+) in a filtrate of an insoluble glucan (poly alpha-1,3-glucan) synthesis reaction was studied.

Isolation and Analysis of Oligosaccharides for Testing Against Alpha-Glucosidase Activity

First, a concentrated filtrate of a glucan synthesis reaction was prepared as per Example 1.

Briefly, oligosaccharides were isolated from the concentrated filtrate by chromatographic separation, and analyzed for glycosidic linkage profile. Chromatographic separation employing a strong acid cation-exchange resin was used to isolate the oligosaccharide fraction of the concentrated filtrate. The physical parameters of the column used for this separation were as follows: FINEX CS11 GC, #227 resin; Na<sup>+</sup> ion form; 5% divinyl benzene (crosslinking); 0.34 mm particle size; 1.64 m bed length; 0.093 m column diameter.

In more detail, the concentrated sugar solution (i.e., concentrated filtrate) described in Table 3 was filtered and diluted to 25 g dry solids/100 g solution using tap water. Prior to addition of this sugar solution to the column resin, the resin was washed with six bed volumes (BV) of sodium chloride solution (three BV at 10 wt % sodium chloride followed by three BV at 5 wt % sodium chloride) to convert the resin to the sodium form. The sugar solution (0.6 L) was then fed to the column, after which the column was eluted using water at a flow rate of 50 mL/min. The run conditions of the chromatographic separation are summarized as follows: 0.6 L feed size, 25 g dry solids/100 g solution, 65° C. column temperature, 50 mL/min flow rate. An oligosaccharide solution was eluted between 11 and 21 minutes. A small amount of salts—indicated by an increase in conductivity—was eluted at the same time. The oligosaccharide fraction thus prepared was analyzed by HPLC to determine its product distribution. In total, the fraction contained >89% of oligosaccharides containing three or more hexose units and less than 1.5% of identifiable mono- and di-saccharides. This fraction was concentrated to a total dry weight of 317 g/L using a thin film evaporator (LCI Corporation, Charlotte, N.C.) followed by rotary evaporation with a ROTAVAPOR (R-151; Buchi, New Castle, Del.). The product distribution of the concentrated fraction as measured by HPLC appears in Table 18.

TABLE 18

Product Distribution of Concentrated Oligosaccharide Fraction											
	Sucrose	Leucrose	Glucose	Fructose	DP2	DP3	DP4	DP5	DP6	DP7	Total
g/L	0.0	2.5	0.0	0.7	31.5	75.9	101.8	62.1	28.9	15.3	316.7
wt %	0.0	0.8	0.0	0.2	9.9	23.9	32.1	19.8	8.5	4.8	100

62

Primary Screening of Aloha-Glucosidases on Glucan Oligomer Hydrolysis

The activities of eleven different alpha-glucosidases (Example 10), as well as the activities of two benchmark enzymes, oligo-1,6-glucosidase (purchased from Megazyme) and transglucosidase (TG L-2000), were individually evaluated against the purified oligosaccharide fraction prepared above (Table 18). Each alpha-glucosidase (dosed at 1 mg/mL) was incubated in a solution containing oligosaccharide substrates (2.9% dry solids) and 2 mM calcium chloride at pH 5.0 at 60° C. Each reaction was quenched after 24 hours of incubation by adding 50 μL of 0.5 M NaOH.

The oligosaccharide/monosaccharide contents of the quenched reactions were determined as follows. A sample of each reaction was diluted 5-fold in water for HPLC analysis. HPLC separation was done using an Agilent 1200 series HPLC system with an AMINEX HPX-42A column (300 mm×7.8 mm) at 85° C. The sample (10 μL) was applied to the HPLC column and separated with an isocratic gradient of MILLI-Q water as the mobile phase at a flow rate of 0.6 mL/min. Oligosaccharide products were detected using a refractive index detector. The numbers provided in Table 19 below reflect the average of peak area percentages (from duplication of each sample) of each DP<sub>n</sub> as a fraction of the total from DP1 to DP7.

TABLE 19

Analysis Glucan Filtrate Oligosaccharides Following Treatment with Alpha-Glucosidase								
Enzyme	SEQ ID NO	DP7 %	DP6 %	DP5 %	DP4 %	DP3 %	DP2 %	DP1 %
Oligo-1,6-glucosidase		0.0	0.6	8.7	27.9	21.9	12.2	28.7
TG L-2000	1	0.0	0.0	0.0	0.0	0.0	3.0	97.0
Nfiglu1	9	0.0	0.0	0.0	0.0	0.0	1.9	98.1
Nerglu1	12	0.0	0.0	0.0	2.8	1.6	7.0	88.6
TauSec098	15	0.0	0.0	0.0	15.3	0.0	4.5	80.2
TauSec099	18	0.0	0.0	0.0	15.7	0.0	0.5	83.7
BloGlu1	20	0.0	0.0	6.7	36.3	35.6	0.0	21.3
BloGlu2	24	0.0	0.7	8.6	31.2	34.9	14.0	10.7
BloGlu3	26	0.0	0.6	8.0	28.4	33.5	13.9	15.6
BspGlu1	30	0.0	0.5	5.2	15.4	16.2	8.2	54.5
BthGlu1	32	0.0	0.0	13.0	12.6	2.0	1.9	70.5
BbrGlu2	36	0.0	0.0	21.5	30.7	24.6	0.0	23.3
BbrGlu5	38	0.0	0.0	8.1	23.8	12.1	15.5	40.5
Blank		0.0	0.0	16.2	38.6	30.4	0.0	14.8

As indicated in Table 19, the oligosaccharide content of the reactions generally shifted toward smaller sized sugars, in comparison with the control reaction (“Blank”) in which there was no enzyme. These results indicate that alpha-

## 63

glucosidase can be used to hydrolyze oligosaccharides comprised within a glucan synthesis reaction and a fraction thereof. Also, given the linkage profile of the oligosaccharides (Examples 3 and 4), and the activity of alpha-glucosidase against various glycosidic linkages in addition to alpha-1,4 linkages (Example 11), it is apparent that alpha-glucosidase can be used to break down oligosaccharides with alpha-1,5 glucosyl-fructose linkages and/or alpha-1,3 and alpha-1,6 glucosyl-glucose linkages. The results provided in Table 19 also suggest that fungal alpha-glucosidases have better hydrolytic activity towards soluble oligosaccharides compared with the bacterial alpha-glucosidases.

#### Confirmation of Alpha-Glucosidase Hydrolytic Activity Toward Oligosaccharide Products of Glucan Synthesis Reactions

Reactions were prepared comprising one or two alpha-glucosidases and a concentrated filtrate obtained from a poly alpha-1,3-glucan synthesis reaction (Table 3). Alpha-glucosidase reactions were dosed with enzyme at 4 ppm, or for blends, each enzyme was used at a 1:1 ratio with a final dosage of 4 ppm. The concentrated filtrate was loaded in each reaction at 10% dry solids. Each reaction further comprised 2 mM calcium chloride at pH 5.0, and was carried out at 60° C. or 65° C. The reactions were quenched by adding 50 µL of 0.5 M NaOH after a 23-hour incubation.

The oligosaccharide/monosaccharide contents of the quenched reactions were determined as follows. A sample of each reaction diluted 25-fold in water for HPLC analysis. HPLC separation was done using an Agilent 1200 series HPLC system with an AMINEX HPX-42A column (300 mm×7.8 mm) at 85° C. The sample (10 µL) was applied to the HPLC column and separated with an isocratic gradient of MILLI-Q water as the mobile phase at a flow rate of 0.6 mL/min. Oligosaccharide products were detected using a refractive index detector. The numbers provided in Table 20 below reflect the average of peak area percentages (from duplication of each sample) of each DP<sub>n</sub> as a fraction of the total. The results provided in Table 20 generally confirm the activity of certain alpha-glucosidases as discussed above regarding the results provided in Table 19.

## 64

Thus, alpha-glucosidase can be used to hydrolyze leucrose and other oligosaccharides present in a fraction (e.g., filtrate) obtained from a glucan synthesis reaction, such as a poly alpha-1,3-glucan synthesis reaction.

## Example 13

#### Isolation of Oligomer/Leucrose Fraction from gtf-S/MUT3325 Reaction

Sucrose (4.50 kg) was dissolved in distilled deionized water to a final total volume of 9.5 L and the resulting solution was heated with stirring at 80° C. for 5 minutes and then cooled to 47° C. With stirring, 500 grams of a crude extract containing 0.6 g/L of gtf-S enzyme (GTF0459, SEQ ID NO:42) and 15.0 mL of a crude extract containing 10 g/L of mutanase (MUT3325, SEQ ID NO:47) was added with stirring (see General Methods for enzyme preparations). The pH of the resulting mixture was immediately adjusted to between pH 5.5 to pH 6.0 by slowly adding a 1:10 (v/v) dilution of 37 wt % HCl with stirring. The reaction temperature and pH were maintained at 47° C. and pH 5.5-6.0, respectively, until sucrose conversion was >95% per HPLC analysis, after which the reaction mixture was immediately adjusted to pH 7.0 to 7.5 and heated to 90° C. for 20 min, then cooled to 25° C. for immediate filtration to remove particulates and precipitate. The resulting filtrate was held at 5° C. prior to IEX/SEC column chromatography using the following resin and conditions: FINEX CS 11 GC SAC in Ca<sup>2+</sup> form, column i.d.=9.3 cm, resin bed height 1.58 m, T=70° C., flow rate=51 mL/min, linear flow rate=0.44 m/h, feed size=0.6 L=171 g, feed RI-DS=25.1 g/100 g, sample interval=3 min. The column fractions collected between 30 min and 67 min were combined, concentrated by evaporation to 66% dissolved solids and analyzed by HPLC as described in the General Methods. Table 21 indicates the oligosaccharide and monosaccharide components of the isolated fraction thus prepared.

TABLE 20

Analysis Glucan Filtrate Sugars Following Treatment with Alpha-Glucosidase												
Temp.	Enzyme	SEQ ID	DP7+ %	DP7 %	DP6 %	DP5 %	DP4 %	DP3 %	DP2 %	Leucrose %	Glucose %	Fructose %
		NO										
60° C.	TG L-2000	1	5.8	0.2	0.4	1.2	1.8	2.0	6.2	0.0	32.0	50.4
	TauSec098	15	5.4	0.0	0.1	0.2	0.3	1.9	3.4	25.4	26.2	37.1
	TauSec099	18	6.0	0.2	0.5	1.2	1.8	2.3	6.8	0.0	30.4	50.8
	TauSec098 + TauSec099	15, 18	6.4	0.1	0.1	0.2	0.5	2.2	8.1	0.0	32.4	50.0
	TauSec098 + TG L-2000	15, 1	6.3	0.1	0.1	0.2	0.4	1.8	8.1	0.0	32.9	50.1
	Blank		5.4	0.2	0.4	1.0	1.6	2.4	2.9	25.1	22.7	38.2
65° C.	TG L-2000	1	6.3	0.1	0.4	1.1	1.7	2.2	6.7	0.0	31.6	49.8
	TauSec098	15	5.8	0.1	0.2	0.2	0.3	1.6	3.3	24.5	27.2	36.7
	TauSec099	18	6.3	0.2	0.5	1.1	1.7	2.1	6.1	0.0	32.3	49.7
	TauSec098 + TauSec099	15, 18	6.7	0.0	0.1	0.2	0.4	1.7	7.8	0.0	34.0	49.2
	TauSec098 + TG L-2000	15, 1	6.8	0.1	0.2	0.2	0.4	1.8	7.6	0.0	33.9	49.1
	Blank		6.0	0.1	0.3	0.9	1.4	2.1	3.0	24.5	23.8	37.7

TABLE 21

Analysis of Oligomer/Leucrose Fraction from gtf-S/MUT3325 Reaction									
DP7+ (% DS)	DP6 (% DS)	DP5 (% DS)	DP4 (% DS)	DP3 (% DS)	DP2 (% DS)	sucrose (% DS)	leucrose (% DS)	glucose (% DS)	fructose (% DS)
0	1.1	2.9	7.2	16.2	12.7	13.5	30.7	7.9	7.9

In this Example, a glucan synthesis reaction was used to produce at least one soluble alpha-glucan product. This soluble product resulted from the concerted action of both a glucosyltransferase (GTF0459, SEQ ID NO:42) and an alpha-glucanohydrolase (MUT3325, SEQ ID NO:47) that were both present in the glucosyltransferase reaction. This Example also demonstrated the preparation of a chromatographic fraction from the glucan synthesis reaction.

This fraction was used in Examples 15 and 16 below to test the activity of alpha-glucosidases thereupon.

#### Example 14

##### Isolation of Oligomer/Leucrose Fraction from Gtf-C Reaction

Sucrose (4.50 kg) was dissolved in distilled deionized water to a final total volume of 9.5 L and the resulting solution was heated with stirring at 80° C. for 5 minutes and then cooled to 47° C. With stirring, 500 grams of a crude extract containing 0.41 g/L of gtf-C enzyme (GTF0088BsT1, SEQ ID NO:45) was added with stirring (see General Methods for enzyme preparation). The pH of the resulting mixture was immediately adjusted to between pH 5.5 to pH 6.0 by slowly adding a 1:10 (v/v) dilution of 37 wt % HCl with stirring. The reaction temperature and pH were maintained at 47° C. and pH 5.5-6.0, respectively, until sucrose conversion was >95% per HPLC analysis, after which the reaction mixture was immediately adjusted to pH 7.0 to 7.5 and heated to 90° C. for 20 min, then cooled to 25° C. for immediate filtration to remove particulates and precipitate. The resulting filtrate held at 5° C. prior to IEX/SEC column chromatography using the following resin and conditions: FINEX CS 11 GC SAC in Ca<sup>2+</sup> form, column i.d.=9.3 cm, resin bed height 1.58 m, T=70° C., flow rate=50 mL/min, linear flow rate=0.44 m/h, feed size=0.6 L=171 g, feed RI-DS=25.8 g/100 g, sample interval=3 min. The column fractions collected between 34 min and 72 min were combined, concentrated by evaporation to 67% dissolved solids and analyzed by HPLC as described in the General Methods. Table 22 indicates the oligosaccharide and monosaccharide components of the isolated fraction thus prepared.

TABLE 22

Analysis of Oligomer/Leucrose Fraction from Gtf-C Reaction									
DP7+ (% DS)	DP6 (% DS)	DP5 (% DS)	DP4 (% DS)	DP3 (% DS)	DP2 (% DS)	sucrose (% DS)	leucrose (% DS)	glucose (% DS)	fructose (% DS)
1.2	0.9	1.5	2.6	5.4	13.2	2.5	59.9	6.9	5.7

In this Example, a glucan synthesis reaction was used to produce at least one soluble alpha-glucan product. This Example also demonstrated the preparation of a chromatographic fraction from a glucan synthesis reaction that produced a soluble alpha-glucan product. This fraction was used in Examples 15 and 16 below to test the activity of alpha-glucosidases thereupon.

#### Example 15

##### Primary Screening of Alpha-Glucosidases Using Oligomer/Leucrose Fractions from Gtf-S/MUT3325 and Gtf-C Reactions

This Example describes using alpha-glucosidase to hydrolyze leucrose and other oligosaccharides present in chromatographic fractions obtained from glucan synthesis reactions that produced soluble alpha-glucan product. Specifically, study was made on the effect of alpha-glucosidases disclosed in Example 10 on the hydrolysis of leucrose and oligosaccharides in the fractions prepared in Examples 13 and 14.

A total of twelve alpha-glucosidases and two benchmark enzymes (oligo-1,6-glucosidase and TG L-2000 transglucosidase) were screened using oligomer/leucrose fractions from gtf-S/MUT3325 (Example 13) and gtf-C (Example 14) reactions as substrate material. All the enzymes (alpha-glucosidases and benchmark enzymes) were dosed at equal protein concentrations. Each alpha-glucosidase (dosed at 100 ppm) was incubated in a solution containing oligomer/leucrose substrates (10% dry solids) and 2 mM calcium chloride at pH 5.5 at 47° C. Each reaction was quenched after 21 hours of incubation by adding 50 µL of 0.5 M NaOH.

The oligosaccharide/monosaccharide contents of the quenched reactions were determined as follows. A sample from each reaction was centrifuged and supernatant therefrom was diluted 25-fold in water for HPLC analysis (General Methods). The percentages reported in Table 23 reflect the average of peak area percentages (from duplicate analyses of each sample) of each DP<sub>n</sub> as a fraction of the total. The results indicate that the fungal alpha-glucosidases had better hydrolytic activity towards glucan oligomers when compared to the bacterial alpha-glucosidases.

TABLE 23

Sugar Composition Analysis of Primary Screening of Alpha-Glucosidases Using Oligomer/Leucrose Fractions from Gtf-S/MUT3325 and Gtf-C reactions												
Substrate	Enzyme	SEQ								Leucrose %	Glucose %	Fructose %
		ID	NO	DP6+ %	DP6 %	DP5 %	DP4 %	DP3 %	DP2 %			
oligomer/ leucrose fraction from Gtf- C reaction	Oligo-1,6- glucosidase		8	0.1	0.2	0.4	1.1	6.8	50.7	19.6	13.1	
	TG L-2000	1	0.7	0.1	0.2	0.5	1.1	5.7	3.8	48.6	39.3	
	Aclglu1	6	0.8	0.1	0.2	0.4	1	4.2	0	51.8	41.6	
	Nfiglu1	9	0.7	0.1	0.2	0.4	1	3.6	0	52.2	41.7	
	Ncrglu1	12	0.7	0	0.2	0.4	1	3.6	4	49.9	40.2	
	BloGlu1	20	0.1	0.3	0.4	0.7	0.6	2.9	20.6	40.7	33.6	
	BloGlu2	24	0.8	0.2	0.6	1	0.9	4	30.9	34	27.5	
	BloGlu3	26	1.1	0.3	0.5	1	1	4	28.8	35.4	28.1	
	BspGlu1	30	0.9	0.1	0.1	0.1	0.3	2.5	27.9	39.5	28.7	
	BthGlu1	32	0.8	0.2	0.5	0.7	0.7	2.9	28.6	37.1	28.4	
	BbrGlu2	36	1.1	0.1	0.6	1.1	1.2	4.7	36.8	30.7	23.7	
	BbrGlu5	38	1.4	0.1	0.2	0.5	0.9	4	44.6	29.1	19.4	
	TauSec098	15	3.1	0.2	0.4	0.7	1.7	4.9	59.3	17.9	11.8	
	TauSec099	18	0.1	0	0.1	0.4	1	3.3	0	51.2	43.6	
	blank		1.1	0.3	0.6	1.5	2.9	7.9	65.4	11.7	8.5	
	oligomer/ leucrose fraction from Gtf- S/ MUT3325 reaction	Oligo-1,6- glucosidase		10.8	0.8	2.8	7.5	15.7	0	27.8	18.9	15.6
		TG L-2000	1	1.4	0.8	2.7	7.3	13.2	2.3	0	43.8	28.6
Aclglu1		6	1.8	0.8	2.5	6.9	12.3	1.2	0	45.6	29	
Nfiglu1		9	1.8	0.8	2.6	7	13.6	0.9	0	44.4	29.1	
Ncrglu1		12	2.1	0.7	2.2	6.6	13.9	0	0	45.2	29.3	
BloGlu1		20	3.5	0.7	2.4	6.5	11.7	2.1	14.8	35.6	22.6	
BloGlu2		24	1.5	0.8	2.6	7	14.6	2.8	24.9	28	17.8	
BloGlu3		26	2	0.8	2.5	6.9	14.1	2.9	22.4	29.9	18.6	
BspGlu1		30	1.7	0.6	1.5	3	3.4	2	18.8	48.3	20.8	
BthGlu1		32	1.4	0.7	2.5	6.2	12.2	2.1	16.3	36.7	21.9	
BbrGlu2		36	1.5	0.8	2.6	7	14.7	3	25	28	17.6	
BbrGlu5		38	2.7	0.7	2.5	5.9	17.2	0	23.9	26.6	20.5	
TauSec098		15	2.9	0	0.1	0.3	1.1	3.6	37.1	41.8	13.1	
TauSec099		18	1.4	0.8	2.8	7.6	16.2	0	0	40.8	30.4	
blank			1.4	0.9	3.1	8.7	19.4	0	37.1	15.6	13.8	

35

As indicated in Table 23, the oligosaccharide content of the reactions generally shifted toward smaller sized sugars, in comparison with the control reactions ("Blank") in which there was no enzyme. These results indicate that alpha-glucosidase can be used to hydrolyze oligosaccharides comprised within a glucan synthesis reaction and a fraction thereof, particularly a chromatographic fraction of a glucan synthesis reaction that produced soluble alpha-glucan product. Also, given the linkage profile of the oligosaccharides (Examples 13 and 14), and the activity of alpha-glucosidase against various glycosidic linkages in addition to alpha-1,4 linkages (Example 11), it is apparent that alpha-glucosidase can be used to break down oligosaccharides with alpha-1,5 glucosyl-fructose linkages and also likely alpha-1,3 and alpha-1,6 glucosyl-glucose linkages. The results provided in Table 23 also suggest that fungal alpha-glucosidases have better hydrolytic activity towards soluble oligosaccharides compared with the bacterial alpha-glucosidases.

Thus, alpha-glucosidase can be used to hydrolyze leucrose and other oligosaccharides present in a fraction (e.g., chromatographic fraction) obtained from a glucan synthesis reaction, such as one that synthesizes a soluble alpha-glucan product.

#### Example 16

##### Select Screening of Alpha-Glucosidases Using Oligomer/Leucrose Fractions from Gtf-S/MUT3325 and Gtf-C Reactions

This Example is further to Example 15, describing the use of alpha-glucosidase to hydrolyze leucrose and other oli-

gosaccharides present in chromatographic fractions obtained from glucan synthesis reactions that produced soluble alpha-glucan product.

Evaluation of alpha-glucosidases that were most active for hydrolysis of oligomer/leucrose fractions from gtf-S/MUT3325 and gtf-C reactions (Example 15) was performed by analyzing sugar compositions resulting in reactions containing enzymes dosed at equal protein concentrations. Incubations of alpha-glucosidases (dosed at 4 ppm; for blends, the ratio of the two enzymes was 1:1 and total dosage was 4 ppm) and oligomer/leucrose substrate (10% ds) were performed at pH 5.5 in the presence of 2 mM calcium chloride at 60° C. and 65° C., respectively. The reactions were quenched by adding 50 µL of 0.5 M NaOH after 23 hours of incubation.

The oligosaccharide/monosaccharide contents of the quenched reactions were determined as follows. A sample from each reaction was centrifuged and supernatant therefrom was diluted 25-fold in water for HPLC analysis (General Methods). The percentages reported in Table 24 (below) reflect the average of peak area percentages (from duplicate analyses of each sample) of each DP<sub>n</sub> as a fraction of the total. The results indicate that TauSec098 was efficacious for hydrolysis of DP2 to DP7 oligomers and TauSec099 outperformed TG L-2000 for leucrose hydrolysis when the incubation was performed at 65° C. The blends of TauSec098 with TauSec099 (or TG L-2000) were effective for hydrolysis of oligomers and leucrose for DP1 production.

Thus, alpha-glucosidase can be used to hydrolyze leucrose and other oligosaccharides present in a fraction (e.g., chromatographic fraction) obtained from a glucan synthesis reaction, such as one that synthesizes a soluble alpha-glucan product.

65

TABLE 24

Sugar Composition Analysis of Select Screening of Alpha-Glucosidases Using Oligomer/Leucrose Fractions from Gtf-S/MUT3325 and Gtf-C reactions														
Temp	Substrate	Enzyme	SEQ ID NO	DP7+ (%)	DP7 (%)	DP6 (%)	DP5 (%)	DP4 (%)	DP3 (%)	DP2 (%)	Leucrose (%)	Glucose (%)	Fructose (%)	
60° C.	oligomer/ leucrose	TG L-2000	1	2.5	0.4	0.5	0.9	1.9	5.3	22.9	18.4	21.4	25.8	
		TauSec098	15	5.4	0.3	0.5	1.0	1.4	2.6	6.3	71.8	0.0	10.7	
	fraction from	TauSec099	18	2.9	0.3	0.5	1.0	1.9	4.7	23.3	21.5	19.7	24.1	
	Gtf-C reaction	TauSec098 + TauSec099	15, 18	2.7	0.3	0.5	0.9	1.4	3.3	19.9	34.9	18.2	17.8	
		TauSec098 + TG L-2000	15, 1	2.7	0.3	0.5	0.8	1.4	4.5	23.7	27.1	18.6	20.3	
		Blank			5.2	0.4	0.6	1.2	1.8	3.2	8.3	70.0	0.0	9.2
	oligomer/ leucrose	TG L-2000	1	4.1	0.3	1.2	3.2	8.1	17.2	13.2	0.0	27.9	24.8	
		TauSec098	15	3.4	0.2	0.0	0.4	1.1	3.5	12.3	32.3	35.3	11.5	
	fraction from	TauSec099	18	4.2	0.0	1.2	3.2	8.2	17.4	15.3	0.0	25.4	25.1	
	Gtf-S/ MUT3325 reaction	TauSec098 + TauSec099	15, 18	3.5	0.2	0.4	0.9	2.3	6.2	16.8	21.2	31.7	16.9	
		TauSec098 + TG L-2000	15, 1	3.2	0.1	0.3	0.7	2.0	6.0	26.0	17.0	29.1	15.6	
		Blank			4.6	0.4	1.2	3.2	7.9	17.5	15.1	36.6	0.0	13.5
	65° C.	oligomer/ leucrose	TG L-2000	1	2.5	0.3	0.5	1.0	1.8	4.9	24.9	26.0	17.4	20.8
			TauSec098	15	2.8	0.4	0.5	1.0	1.4	2.6	6.6	73.6	0.0	11.1
fraction from		TauSec099	18	2.2	0.3	0.4	1.0	2.0	4.9	23.2	17.4	21.6	27.0	
Gtf-C reaction		TauSec098 + TauSec099	15, 18	4.5	0.3	0.5	0.9	1.4	3.4	20.3	28.6	20.1	20.1	
		TauSec098 + TG L-2000	15, 1	5.1	0.3	0.5	0.9	1.2	2.9	21.1	34.4	18.0	15.7	
		Blank			7.0	0.4	0.7	1.3	1.8	3.2	7.9	68.4	0.0	9.4
oligomer/ leucrose		TG L-2000	1	2.9	0.2	1.1	3.1	8.1	18.0	11.7	16.5	19.3	19.0	
		TauSec098	15	2.6	0.0	0.1	0.3	0.9	3.3	12.0	33.1	36.6	11.1	
fraction from		TauSec099	18	4.4	0.0	1.2	3.1	7.8	16.1	14.4	0.0	27.4	25.5	
Gtf-S/ MUT3325 reaction		TauSec098 + TauSec099	15, 18	3.9	0.2	0.4	0.8	2.1	5.7	16.2	19.4	33.7	17.6	
		TauSec098 + TG L-2000	15, 1	3.7	0.2	0.3	0.7	1.8	5.0	24.9	20.5	29.4	13.6	
		Blank			3.1	0.6	1.1	2.5	6.3	13.6	13.0	31.1	17.0	11.8

## SEQUENCE LISTING

&lt;160&gt; NUMBER OF SEQ ID NOS: 47

&lt;210&gt; SEQ ID NO 1

&lt;211&gt; LENGTH: 965

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Aspergillus niger

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Asp Pro Gln Ala Ala Asp Ala Gln Ser Val Cys Pro Gly Tyr Lys Ala  
35 40 45

Ser Lys Val Gln His Asn Ser Arg Gly Phe Thr Ala Ser Leu Gln Leu  
50 55 60

Ala Gly Arg Pro Cys Asn Val Tyr Gly Thr Asp Val Glu Ser Leu Thr  
65 70 75 80

Leu Ser Val Glu Tyr Gln Asp Ser Asp Arg Leu Asn Ile Gln Ile Leu  
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Pro Thr His Val Asp Ser Thr Asn Ala Ser Trp Tyr Phe Leu Ser Glu  
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Asn Leu Val Pro Arg Pro Lys Ala Ser Leu Asn Ala Ser Val Ser Gln  
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 165 170 175  
 Glu Glu Tyr Asn Leu Tyr Gly Leu Gly Glu His Ile Thr Gln Phe Arg  
 180 185 190  
 Leu Gln Arg Asn Ala Asn Leu Thr Ile Tyr Pro Ser Asp Asp Gly Thr  
 195 200 205  
 Pro Ile Asp Gln Asn Leu Tyr Gly Gln His Pro Phe Tyr Leu Asp Thr  
 210 215 220  
 Arg Tyr Tyr Lys Gly Asp Arg Gln Asn Gly Ser Tyr Ile Pro Val Lys  
 225 230 235 240  
 Ser Ser Glu Ala Asp Ala Ser Gln Asp Tyr Ile Ser Leu Ser His Gly  
 245 250 255  
 Val Phe Leu Arg Asn Ser His Gly Leu Glu Ile Leu Leu Arg Ser Gln  
 260 265 270  
 Lys Leu Ile Trp Arg Thr Leu Gly Gly Gly Ile Asp Leu Thr Phe Tyr  
 275 280 285  
 Ser Gly Pro Ala Pro Ala Asp Val Thr Arg Gln Tyr Leu Thr Ser Thr  
 290 295 300  
 Val Gly Leu Pro Ala Met Gln Gln Tyr Asn Thr Leu Gly Phe His Gln  
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 Cys Arg Trp Gly Tyr Asn Asn Trp Ser Asp Leu Ala Asp Val Val Ala  
 325 330 335  
 Asn Phe Glu Lys Phe Glu Ile Pro Leu Glu Tyr Ile Trp Thr Asp Ile  
 340 345 350  
 Asp Tyr Met His Gly Tyr Arg Asn Phe Asp Asn Asp Gln His Arg Phe  
 355 360 365  
 Ser Tyr Ser Glu Gly Asp Glu Phe Leu Ser Lys Leu His Glu Ser Gly  
 370 375 380  
 Arg Tyr Tyr Val Pro Ile Val Asp Ala Ala Leu Tyr Ile Pro Asn Pro  
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 Glu Asn Ala Ser Asp Ala Tyr Ala Thr Tyr Asp Arg Gly Ala Ala Asp  
 405 410 415  
 Asp Val Phe Leu Lys Asn Pro Asp Gly Ser Leu Tyr Ile Gly Ala Val  
 420 425 430  
 Trp Pro Gly Tyr Thr Val Phe Pro Asp Trp His His Pro Lys Ala Val  
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 Asp Phe Trp Ala Asn Glu Leu Val Ile Trp Ser Lys Lys Val Ala Phe  
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 Asp Gly Val Trp Tyr Asp Met Ser Glu Val Ser Ser Phe Cys Val Gly  
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 Ser Cys Gly Thr Gly Asn Leu Thr Leu Asn Pro Ala His Pro Ser Phe  
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 Leu Leu Pro Gly Glu Pro Gly Asp Ile Ile Tyr Asp Tyr Pro Glu Ala  
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 Phe Asn Ile Thr Asn Ala Thr Glu Ala Ala Ser Ala Ser Ala Gly Ala  
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 Ser Ser Gln Ala Ala Ala Thr Ala Thr Thr Thr Ser Thr Ser Val Ser  
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Val His Gly Leu Tyr Gly His Gln Gly Leu Asn Ala Thr Tyr Gln Gly  
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Leu Leu Glu Val Trp Ser His Lys Arg Arg Pro Phe Ile Ile Gly Arg  
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Ser Thr Phe Ala Gly Ser Gly Lys Trp Ala Gly His Trp Gly Gly Asp  
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Asn Tyr Ser Lys Trp Trp Ser Met Tyr Tyr Ser Ile Ser Gln Ala Leu  
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Ser Phe Ser Leu Phe Asp Ile Pro Met Phe Gly Ala Asp Thr Cys Gly  
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Phe Asn Gly Asn Ser Asp Glu Glu Leu Cys Asn Arg Trp Met Gln Leu  
 675 680 685

Ser Ala Phe Phe Pro Phe Tyr Arg Asn His Asn Glu Leu Ser Thr Ile  
 690 695 700

Pro Gln Glu Pro Tyr Arg Trp Ala Ser Val Ile Glu Ala Thr Lys Ser  
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Ala Met Arg Ile Arg Tyr Ala Ile Leu Pro Tyr Phe Tyr Thr Leu Phe  
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Asp Leu Ala His Thr Thr Gly Ser Thr Val Met Arg Ala Leu Ser Trp  
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Glu Phe Pro Asn Asp Pro Thr Leu Ala Ala Val Glu Thr Gln Phe Met  
 755 760 765

Val Gly Pro Ala Ile Met Val Val Pro Val Leu Glu Pro Leu Val Asn  
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Thr Val Lys Gly Val Phe Pro Gly Val Gly His Gly Glu Val Trp Tyr  
 785 790 795 800

Asp Trp Tyr Thr Gln Ala Ala Val Asp Ala Lys Pro Gly Val Asn Thr  
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Thr Ile Ser Ala Pro Leu Gly His Ile Pro Val Tyr Val Arg Gly Gly  
 820 825 830

Asn Ile Leu Pro Met Gln Glu Pro Ala Leu Thr Thr Arg Glu Ala Arg  
 835 840 845

Gln Thr Pro Trp Ala Leu Leu Ala Ala Leu Gly Ser Asn Gly Thr Ala  
 850 855 860

Ser Gly Gln Leu Tyr Leu Asp Asp Gly Glu Ser Ile Tyr Pro Asn Ala  
 865 870 875 880

Thr Leu His Val Asp Phe Thr Ala Ser Arg Ser Ser Leu Arg Ser Ser  
 885 890 895

Ala Gln Gly Arg Trp Lys Glu Arg Asn Pro Leu Ala Asn Val Thr Val  
 900 905 910

Leu Gly Val Asn Lys Glu Pro Ser Ala Val Thr Leu Asn Gly Gln Ala  
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Val Phe Pro Gly Ser Val Thr Tyr Asn Ser Thr Ser Gln Val Leu Phe  
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Trp Val Leu Glu Trp  
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<210> SEQ ID NO 2  
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<212> TYPE: PRT  
<213> ORGANISM: Trichoderma reesei

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Ala Gly Ala Val Ile Ala Ser Pro Ser Thr Ile Asp Pro Asp Tyr Tyr  
35 40 45  
Tyr Met Trp Thr Arg Asp Ser Ala Leu Val Phe Lys Asn Leu Ile Asp  
50 55 60  
Arg Phe Thr Glu Thr Tyr Asp Ala Gly Leu Gln Arg Arg Ile Glu Gln  
65 70 75 80  
Tyr Ile Thr Ala Gln Val Thr Leu Gln Gly Leu Ser Asn Pro Ser Gly  
85 90 95  
Ser Leu Ala Asp Gly Ser Gly Leu Gly Glu Pro Lys Phe Glu Leu Thr  
100 105 110  
Leu Lys Pro Phe Thr Gly Asn Trp Gly Arg Pro Gln Arg Asp Gly Pro  
115 120 125  
Ala Leu Arg Ala Ile Ala Leu Ile Gly Tyr Ser Lys Trp Leu Ile Asn  
130 135 140  
Asn Asn Tyr Gln Ser Thr Val Ser Asn Val Ile Trp Pro Ile Val Arg  
145 150 155 160  
Asn Asp Leu Asn Tyr Val Ala Gln Tyr Trp Asn Gln Thr Gly Phe Asp  
165 170 175  
Leu Trp Glu Glu Val Asn Gly Ser Ser Phe Phe Thr Val Ala Asn Gln  
180 185 190  
His Arg Ala Leu Val Glu Gly Ala Thr Leu Ala Ala Thr Leu Gly Gln  
195 200 205  
Ser Gly Ser Ala Tyr Ser Ser Val Ala Pro Gln Val Leu Cys Phe Leu  
210 215 220  
Gln Arg Phe Trp Val Ser Ser Gly Gly Tyr Val Asp Ser Asn Ile Asn  
225 230 235 240  
Thr Asn Glu Gly Arg Thr Gly Lys Asp Val Asn Ser Val Leu Thr Ser  
245 250 255  
Ile His Thr Phe Asp Pro Asn Leu Gly Cys Asp Ala Gly Thr Phe Gln  
260 265 270  
Pro Cys Ser Asp Lys Ala Leu Ser Asn Leu Lys Val Val Val Asp Ser  
275 280 285  
Phe Arg Ser Ile Tyr Gly Val Asn Lys Gly Ile Pro Ala Gly Ala Ala  
290 295 300  
Val Ala Ile Gly Arg Tyr Ala Glu Asp Val Tyr Tyr Asn Gly Asn Pro  
305 310 315 320  
Trp Tyr Leu Ala Thr Phe Ala Ala Ala Glu Gln Leu Tyr Asp Ala Ile  
325 330 335  
Tyr Val Trp Lys Lys Thr Gly Ser Ile Thr Val Thr Ala Thr Ser Leu  
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Ala Phe Phe Gln Glu Leu Val Pro Gly Val Thr Ala Gly Thr Tyr Ser  
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Ser Ser Ser Ser Thr Phe Thr Asn Ile Ile Asn Ala Val Ser Thr Tyr  
 370 375 380

Ala Asp Gly Phe Leu Ser Glu Ala Ala Lys Tyr Val Pro Ala Asp Gly  
 385 390 395 400

Ser Leu Ala Glu Gln Phe Asp Arg Asn Ser Gly Thr Pro Leu Ser Ala  
 405 410 415

Leu His Leu Thr Trp Ser Tyr Ala Ser Phe Leu Thr Ala Thr Ala Arg  
 420 425 430

Arg Ala Gly Ile Val Pro Pro Ser Trp Ala Asn Ser Ser Ala Ser Thr  
 435 440 445

Ile Pro Ser Thr Cys Ser Gly Ala Ser Val Val Gly Ser Tyr Ser Arg  
 450 455 460

Pro Thr Ala Thr Ser Phe Pro Pro Ser Gln Thr Pro Lys Pro Gly Val  
 465 470 475 480

Pro Ser Gly Thr Pro Tyr Thr Pro Leu Pro Cys Ala Thr Pro Thr Ser  
 485 490 495

Val Ala Val Thr Phe His Glu Leu Val Ser Thr Gln Phe Gly Gln Thr  
 500 505 510

Val Lys Val Ala Gly Asn Ala Ala Ala Leu Gly Asn Trp Ser Thr Ser  
 515 520 525

Ala Ala Val Ala Leu Asp Ala Val Asn Tyr Ala Asp Asn His Pro Leu  
 530 535 540

Trp Ile Gly Thr Val Asn Leu Glu Ala Gly Asp Val Val Glu Tyr Lys  
 545 550 555 560

Tyr Ile Asn Val Gly Gln Asp Gly Ser Val Thr Trp Glu Ser Asp Pro  
 565 570 575

Asn His Thr Tyr Thr Val Pro Ala Val Ala Cys Val Thr Gln Val Val  
 580 585 590

Lys Glu Asp Thr Trp Gln Ser  
 595

&lt;210&gt; SEQ ID NO 3

&lt;211&gt; LENGTH: 1477

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Streptococcus salivarius

&lt;400&gt; SEQUENCE: 3

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 20 25 30

Arg Thr Asn Thr Lys Glu Ala Asp Val Leu Thr Pro Ala Lys Glu Thr  
 35 40 45

Asn Ala Val Glu Thr Ala Thr Thr Thr Asn Thr Gln Ala Thr Ala Glu  
 50 55 60

Ala Ala Thr Thr Ala Thr Thr Ala Asp Val Ala Val Ala Ala Val Pro  
 65 70 75 80

Asn Lys Glu Ala Val Thr Thr Asp Ala Pro Ala Val Thr Thr Glu  
 85 90 95

Lys Ala Glu Glu Gln Pro Ala Thr Val Lys Ala Glu Val Val Asn Thr  
 100 105 110

Glu Val Lys Ala Pro Glu Ala Ala Leu Lys Asp Ser Glu Val Glu Ala

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Val	Asn	Glu	Asp	Gly	Ser	His	Lys	Glu	Asn	Phe	Ala	Ile	Thr	Val	Asn
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Gly	Gln	Leu	Leu	Tyr	Phe	Gly	Lys	Asp	Gly	Ala	Leu	Thr	Ser	Ser	Ser
				165					170					175	
Thr	Tyr	Ser	Phe	Thr	Pro	Gly	Thr	Thr	Asn	Ile	Val	Asp	Gly	Phe	Ser
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Ile	Asn	Asn	Arg	Ala	Tyr	Asp	Ser	Ser	Glu	Ala	Ser	Phe	Glu	Leu	Ile
	195					200						205			
Asp	Gly	Tyr	Leu	Thr	Ala	Asp	Ser	Trp	Tyr	Arg	Pro	Ala	Ser	Ile	Ile
	210					215					220				
Lys	Asp	Gly	Val	Thr	Trp	Gln	Ala	Ser	Thr	Ala	Glu	Asp	Phe	Arg	Pro
225					230					235					240
Leu	Leu	Met	Ala	Trp	Trp	Pro	Asn	Val	Asp	Thr	Gln	Val	Asn	Tyr	Leu
				245					250					255	
Asn	Tyr	Met	Ser	Lys	Val	Phe	Asn	Leu	Asp	Ala	Lys	Tyr	Ser	Ser	Thr
			260					265					270		
Asp	Lys	Gln	Glu	Thr	Leu	Lys	Val	Ala	Ala	Lys	Asp	Ile	Gln	Ile	Lys
		275					280					285			
Ile	Glu	Gln	Lys	Ile	Gln	Ala	Glu	Lys	Ser	Thr	Gln	Trp	Leu	Arg	Glu
	290					295					300				
Thr	Ile	Ser	Ala	Phe	Val	Lys	Thr	Gln	Pro	Gln	Trp	Asn	Lys	Glu	Thr
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Glu	Asn	Tyr	Ser	Lys	Gly	Gly	Gly	Glu	Asp	His	Leu	Gln	Gly	Gly	Ala
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Leu	Leu	Tyr	Val	Asn	Asp	Ser	Arg	Thr	Pro	Trp	Ala	Asn	Ser	Asp	Tyr
			340					345					350		
Arg	Arg	Leu	Asn	Arg	Thr	Ala	Thr	Asn	Gln	Thr	Gly	Thr	Ile	Asp	Lys
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Ser	Ile	Leu	Asp	Glu	Gln	Ser	Asp	Pro	Asn	His	Met	Gly	Gly	Phe	Asp
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Phe	Leu	Leu	Ala	Asn	Asp	Val	Asp	Leu	Ser	Asn	Pro	Val	Val	Gln	Ala
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Glu	Gln	Leu	Asn	Gln	Ile	His	Tyr	Leu	Met	Asn	Trp	Gly	Ser	Ile	Val
				405					410					415	
Met	Gly	Asp	Lys	Asp	Ala	Asn	Phe	Asp	Gly	Ile	Arg	Val	Asp	Ala	Val
			420					425					430		
Asp	Asn	Val	Asp	Ala	Asp	Met	Leu	Gln	Leu	Tyr	Thr	Asn	Tyr	Phe	Arg
		435				440					445				
Glu	Tyr	Tyr	Gly	Val	Asn	Lys	Ser	Glu	Ala	Asn	Ala	Leu	Ala	His	Ile
	450					455					460				
Ser	Val	Leu	Glu	Ala	Trp	Ser	Leu	Asn	Asp	Asn	His	Tyr	Asn	Asp	Lys
465				470					475					480	
Thr	Asp	Gly	Ala	Ala	Leu	Ala	Met	Glu	Asn	Lys	Gln	Arg	Leu	Ala	Leu
				485					490					495	
Leu	Phe	Ser	Leu	Ala	Lys	Pro	Ile	Lys	Glu	Arg	Thr	Pro	Ala	Val	Ser
			500					505					510		
Pro	Leu	Tyr	Asn	Asn	Thr	Phe	Asn	Thr	Thr	Gln	Arg	Asp	Glu	Lys	Thr
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Asp	Trp	Ile	Asn	Lys	Asp	Gly	Ser	Lys	Ala	Tyr	Asn	Glu	Asp	Gly	Thr
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Val Lys Gln Ser Thr Ile Gly Lys Tyr Asn Glu Lys Tyr Gly Asp Ala  
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 Ser Gly Asn Tyr Val Phe Ile Arg Ala His Asp Asn Asn Val Gln Asp  
 565 570 575  
 Ile Ile Ala Glu Ile Ile Lys Lys Glu Ile Asn Pro Lys Ser Asp Gly  
 580 585 590  
 Phe Thr Ile Thr Asp Ala Glu Met Lys Gln Ala Phe Glu Ile Tyr Asn  
 595 600 605  
 Lys Asp Met Leu Ser Ser Asp Lys Lys Tyr Thr Leu Asn Asn Ile Pro  
 610 615 620  
 Ala Ala Tyr Ala Val Met Leu Gln Asn Met Glu Thr Ile Thr Arg Val  
 625 630 635 640  
 Tyr Tyr Gly Asp Leu Tyr Thr Asp Asp Gly His Tyr Met Glu Thr Lys  
 645 650 655  
 Ser Pro Tyr Tyr Asp Thr Ile Val Asn Leu Met Lys Ser Arg Ile Lys  
 660 665 670  
 Tyr Val Ser Gly Gly Gln Ala Gln Arg Ser Tyr Trp Leu Pro Thr Asp  
 675 680 685  
 Gly Lys Met Asp Asn Ser Asp Val Glu Leu Tyr Arg Thr Asn Glu Val  
 690 695 700  
 Tyr Thr Ser Val Arg Tyr Gly Lys Asp Ile Met Thr Ala Asn Asp Thr  
 705 710 715 720  
 Glu Gly Ser Lys Tyr Ser Arg Thr Ser Gly Gln Val Thr Leu Val Ala  
 725 730 735  
 Asn Asn Pro Lys Leu Asn Leu Asp Gln Ser Ala Lys Leu Asn Val Glu  
 740 745 750  
 Met Gly Lys Ile His Ala Asn Gln Lys Tyr Arg Ala Leu Ile Val Gly  
 755 760 765  
 Thr Ala Asp Gly Ile Lys Asn Phe Thr Ser Asp Ala Asp Ala Ile Ala  
 770 775 780  
 Ala Gly Tyr Val Lys Glu Thr Asp Ser Asn Gly Val Leu Thr Phe Gly  
 785 790 795 800  
 Ala Asn Asp Ile Lys Gly Tyr Glu Thr Phe Asp Met Ser Gly Phe Val  
 805 810 815  
 Ala Val Trp Val Pro Val Gly Ala Ser Asp Asn Gln Asp Ile Arg Val  
 820 825 830  
 Ala Pro Ser Thr Glu Ala Lys Lys Glu Gly Glu Leu Thr Leu Lys Ala  
 835 840 845  
 Thr Glu Ala Tyr Asp Ser Gln Leu Ile Tyr Glu Gly Phe Ser Asn Phe  
 850 855 860  
 Gln Thr Ile Pro Asp Gly Ser Asp Pro Ser Val Tyr Thr Asn Arg Lys  
 865 870 875 880  
 Ile Ala Glu Asn Val Asp Leu Phe Lys Ser Trp Gly Val Thr Ser Phe  
 885 890 895  
 Glu Met Ala Pro Gln Phe Val Ser Ala Asp Asp Gly Thr Phe Leu Asp  
 900 905 910  
 Ser Val Ile Gln Asn Gly Tyr Ala Phe Ala Asp Arg Tyr Asp Leu Ala  
 915 920 925  
 Met Ser Lys Asn Asn Lys Tyr Gly Ser Lys Glu Asp Leu Arg Asp Ala  
 930 935 940  
 Leu Lys Ala Leu His Lys Ala Gly Ile Gln Ala Ile Ala Asp Trp Val  
 945 950 955 960

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Pro Asp Gln Ile Tyr Gln Leu Pro Gly Lys Glu Val Val Thr Ala Thr  
                           965                          970                          975

Arg Thr Asp Gly Ala Gly Arg Lys Ile Ala Asp Ala Ile Ile Asp His  
                           980                          985                          990

Ser Leu Tyr Val Ala Asn Ser Lys Ser Ser Gly Lys Asp Tyr Gln Ala  
                           995                          1000                          1005

Lys Tyr Gly Gly Glu Phe Leu Ala Glu Leu Lys Ala Lys Tyr Pro  
           1010                          1015                          1020

Glu Met Phe Lys Val Asn Met Ile Ser Thr Gly Lys Pro Ile Asp  
           1025                          1030                          1035

Asp Ser Val Lys Leu Lys Gln Trp Lys Ala Glu Tyr Phe Asn Gly  
           1040                          1045                          1050

Thr Asn Val Leu Glu Arg Gly Val Gly Tyr Val Leu Ser Asp Glu  
           1055                          1060                          1065

Ala Thr Gly Lys Tyr Phe Thr Val Thr Lys Glu Gly Asn Phe Ile  
           1070                          1075                          1080

Pro Leu Gln Leu Thr Gly Lys Glu Lys Val Ile Thr Gly Phe Ser  
           1085                          1090                          1095

Ser Asp Gly Lys Gly Ile Thr Tyr Phe Gly Thr Ser Gly Thr Gln  
           1100                          1105                          1110

Ala Lys Ser Ala Phe Val Thr Phe Asn Gly Asn Thr Tyr Tyr Phe  
           1115                          1120                          1125

Asp Ala Arg Gly His Met Val Thr Asn Ser Glu Tyr Ser Pro Asn  
           1130                          1135                          1140

Gly Lys Asp Val Tyr Arg Phe Leu Pro Asn Gly Ile Met Leu Ser  
           1145                          1150                          1155

Asn Ala Phe Tyr Ile Asp Ala Asn Gly Asn Thr Tyr Leu Tyr Asn  
           1160                          1165                          1170

Ser Lys Gly Gln Met Tyr Lys Gly Gly Tyr Thr Lys Phe Asp Val  
           1175                          1180                          1185

Ser Glu Thr Asp Lys Asp Gly Lys Glu Ser Lys Val Val Lys Phe  
           1190                          1195                          1200

Arg Tyr Phe Thr Asn Glu Gly Val Met Ala Lys Gly Val Thr Val  
           1205                          1210                          1215

Ile Asp Gly Phe Thr Gln Tyr Phe Gly Glu Asp Gly Phe Gln Ala  
           1220                          1225                          1230

Lys Asp Lys Leu Val Thr Phe Lys Gly Lys Thr Tyr Tyr Phe Asp  
           1235                          1240                          1245

Ala His Thr Gly Asn Gly Ile Lys Asp Thr Trp Arg Asn Ile Asn  
           1250                          1255                          1260

Gly Lys Trp Tyr Tyr Phe Asp Ala Asn Gly Val Ala Ala Thr Gly  
           1265                          1270                          1275

Ala Gln Val Ile Asn Gly Gln Lys Leu Tyr Phe Asn Glu Asp Gly  
           1280                          1285                          1290

Ser Gln Val Lys Gly Gly Val Val Lys Asn Ala Asp Gly Thr Tyr  
           1295                          1300                          1305

Ser Lys Tyr Lys Glu Gly Phe Gly Glu Leu Val Thr Asn Glu Phe  
           1310                          1315                          1320

Phe Thr Thr Asp Gly Asn Val Trp Tyr Tyr Ala Gly Ala Asn Gly  
           1325                          1330                          1335

Lys Thr Val Thr Gly Ala Gln Val Ile Asn Gly Gln His Leu Tyr  
           1340                          1345                          1350

Phe Asn Ala Asp Gly Ser Gln Val Lys Gly Gly Val Val Lys Asn

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1355	1360	1365
Ala Asp Gly Thr Tyr Ser Lys Tyr Asn Ala Ser Thr Gly Glu Arg 1370 1375 1380		
Leu Thr Asn Glu Phe Phe Thr Thr Gly Asp Asn Asn Trp Tyr Tyr 1385 1390 1395		
Ile Gly Ala Asn Gly Lys Ser Val Thr Gly Glu Val Lys Ile Gly 1400 1405 1410		
Asp Asp Thr Tyr Phe Phe Ala Lys Asp Gly Lys Gln Val Lys Gly 1415 1420 1425		
Gln Thr Val Ser Ala Gly Asn Gly Arg Ile Ser Tyr Tyr Tyr Gly 1430 1435 1440		
Asp Ser Gly Lys Arg Ala Val Ser Thr Trp Ile Glu Ile Gln Pro 1445 1450 1455		
Gly Val Tyr Val Tyr Phe Asp Lys Asn Gly Leu Ala Tyr Pro Pro 1460 1465 1470		
Arg Val Leu Asn 1475		

&lt;210&gt; SEQ ID NO 4

&lt;211&gt; LENGTH: 3147

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Aspergillus clavatus

&lt;400&gt; SEQUENCE: 4

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gcggttcaact cgtttgctcc tagtacgacg gcaaccgcag cttactcgca gcatactttg      120
ccttcgtcca ttgacaatgg ggctcagttg attgccaata tcgatgaccc cttggcagtg      180
aatgcgcagt ccgtttgctc aggctataag gcctcggatg tgcaacagac atcacggggt      240
ttcaccgcca gcctgcagct tgcggggcag ccgtgcaatg cctatgggat tgacgttgac      300
tcgctgagtc tctcgggtga agttctggcg aaggatcgct tgaatatcca gattgtccct      360
accatgtgg atagctctaa tgccctcttg tacattcttc cagaggaccg ggtaccocaaa      420
gcgcaagcat ctgccgacgc ctccgtctct caaagtgact tcgagatcga gtggtccaat      480
gaccctcgt tcaatatcaa gatcatccgg aaggcaactg gggatgccct gtttgacaca      540
gccgattcgg tcttggctct tcagaatcag ttcacgagat ttgtgtctgc tttgcctgag      600
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atgaatcctt gatctcgttg gctcacaatg gaccaatagc aacatctatg gacagcatcc      780
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caccgatgat gccgatgcgt ctggagatta tgagtccttc tcccacggcg tgttcctgag      900
aaatgcacat ggacaggagg ttattctgca gtctcgcaac attacatggc ggacaatcgg      960
tggaagcatc gattgaacct tctactcagg tcccactcag gcagatgtca ccaagagcta     1020
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tcgataatga ccaagtccat tccccctacg atgaaggcga ggagttcctc gacagacttc     1320
acaaatcagg gcgtcactgg gtccccatcg tcgactccgc catctacatc cctaaccctg     1380

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acaatgcgtc tgacgcgtaa gtcattgtgt cttatttggc cagcttttca aacagatgct 1440
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aagaaccctg atggcagcct ttatatcgga gctgtatggc cgggattcac agtcttcocct 1560
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gagcctgccc gcgtcacct caaccgccgc agggcccccc ccgagtcggt ggagtataat 3060
gcgacctccc aggttttgac cgtgagcgga ctgcagaagc tgacgccccg cggggcttgg 3120
gctgaggatt ggattctgaa gtggttaa 3147

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&lt;210&gt; SEQ ID NO 5

&lt;211&gt; LENGTH: 990

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Aspergillus clavatus*

&lt;400&gt; SEQUENCE: 5

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Met Ala Ser Leu Val Gly Leu Leu Ala Ser Ala Trp Leu Leu Pro Ser
1           5           10           15

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Thr Tyr Gly Ala Ala Val His Ser Phe Ala Pro Ser Thr Thr Ala Thr
20           25           30

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Ala Ala Tyr Ser Gln His Thr Leu Pro Ser Ser Ile Asp Asn Gly Ala
35           40           45

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Gln Leu Ile Ala Asn Ile Asp Asp Pro Leu Ala Val Asn Ala Gln Ser

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Asp Gly Ser Ser Val Asn Pro Ser Ser Thr Leu Asn Val Glu Phe Ala  
 900 905 910

Ala Thr His Ser Ser Ile Lys Val Ser Ala Lys Gly Asp Trp Arg Glu  
 915 920 925

Lys Asn Ser Leu Asp Ser Val Thr Val Leu Gly Val Ala Lys Glu Pro  
 930 935 940

Ala Arg Val Thr Phe Asn Arg Arg Arg Val Pro Pro Glu Ser Val Glu  
 945 950 955 960

Tyr Asn Ala Thr Ser Gln Val Leu Thr Val Ser Gly Leu Gln Lys Leu  
 965 970 975

Thr Pro Arg Gly Ala Trp Ala Glu Asp Trp Ile Leu Lys Trp  
 980 985 990

<210> SEQ ID NO 6  
 <211> LENGTH: 971  
 <212> TYPE: PRT  
 <213> ORGANISM: *Aspergillus clavatus*

<400> SEQUENCE: 6

Ala Ala Val His Ser Phe Ala Pro Ser Thr Thr Ala Thr Ala Ala Tyr  
 1 5 10 15

Ser Gln His Thr Leu Pro Ser Ser Ile Asp Asn Gly Ala Gln Leu Ile  
 20 25 30

Ala Asn Ile Asp Asp Pro Leu Ala Val Asn Ala Gln Ser Val Cys Pro  
 35 40 45

Gly Tyr Lys Ala Ser Asp Val Gln Gln Thr Ser Arg Gly Phe Thr Ala  
 50 55 60

Ser Leu Gln Leu Ala Gly Glu Pro Cys Asn Ala Tyr Gly Ile Asp Val  
 65 70 75 80

Asp Ser Leu Ser Leu Ser Val Glu Val Leu Ala Lys Asp Arg Leu Asn  
 85 90 95

Ile Gln Ile Val Pro Thr His Val Asp Ser Ser Asn Ala Ser Trp Tyr  
 100 105 110

Ile Leu Pro Glu Asp Arg Val Pro Lys Ala Gln Ala Ser Ala Asp Ala  
 115 120 125

Ser Val Ser Gln Ser Asp Phe Glu Ile Glu Trp Ser Asn Asp Pro Ser  
 130 135 140

Phe Asn Ile Lys Ile Ile Arg Lys Ala Thr Gly Asp Ala Leu Phe Asp  
 145 150 155 160

Thr Ala Asp Ser Val Leu Val Phe Gln Asn Gln Phe Ile Glu Phe Val  
 165 170 175

Ser Ala Leu Pro Glu Gly Tyr Asn Leu Tyr Gly Leu Gly Glu Arg Met  
 180 185 190

Ala Gln Leu Arg Leu Leu Arg Asn Ala Thr Leu Thr Thr Tyr Ala Ala  
 195 200 205

Asp Val Gly Asp Pro Ile Asp Asp Asn Ile Tyr Gly Gln His Pro Phe  
 210 215 220

Tyr Leu Asp Thr Arg Tyr Tyr Thr Lys Asp Ala Asn Gly Ser Tyr Ser  
 225 230 235 240

Leu Val Asn Thr Asp Asp Ala Asp Ala Ser Gly Asp Tyr Glu Ser Phe  
 245 250 255

Ser His Gly Val Phe Leu Arg Asn Ala His Gly Gln Glu Val Ile Leu  
 260 265 270

Gln Ser Arg Asn Ile Thr Trp Arg Thr Ile Gly Gly Ser Ile Asp Leu  
 275 280 285

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Thr Phe Tyr Ser Gly Pro Thr Gln Ala Asp Val Thr Lys Ser Tyr Gln  
 290 295 300  
 Leu Thr Thr Ile Gly Leu Pro Ala Met Gln Gln Tyr Ser Ala Leu Gly  
 305 310 315 320  
 Phe His Gln Cys Arg Trp Gly Tyr Arg Ser Trp Ser Glu Leu Glu Glu  
 325 330 335  
 Val Val Asn Thr Phe Glu Gln Phe Glu Ile Pro Leu Glu Tyr Ile Trp  
 340 345 350  
 Thr Asp Ile Asp Tyr Met Arg Gly Tyr Arg Asp Phe Asp Asn Asp Gln  
 355 360 365  
 Val His Phe Pro Tyr Asp Glu Gly Glu Glu Phe Leu Asp Arg Leu His  
 370 375 380  
 Lys Ser Gly Arg His Trp Val Pro Ile Val Asp Ser Ala Ile Tyr Ile  
 385 390 395 400  
 Pro Asn Pro Asp Asn Ala Ser Asp Ala Tyr Asp Thr Tyr Ala Arg Gly  
 405 410 415  
 Ala Lys Asp Asp Val Phe Ile Lys Asn Pro Asp Gly Ser Leu Tyr Ile  
 420 425 430  
 Gly Ala Val Trp Pro Gly Phe Thr Val Phe Pro Asp Trp His Asn Pro  
 435 440 445  
 Lys Ala Ala Glu Trp Trp Ser Asn Glu Leu Val Thr Trp Phe Glu Lys  
 450 455 460  
 Val Gln Tyr Asp Gly Ile Trp Ile Asp Met Ser Glu Val Ser Ser Phe  
 465 470 475 480  
 Cys Val Gly Ser Cys Gly Thr Gly Asn Leu His Leu Asn Pro Ala His  
 485 490 495  
 Pro Pro Phe Gln Leu Pro Gly Glu Pro Gly Asn Ile Glu Tyr Thr Tyr  
 500 505 510  
 Pro Glu Ala Phe Asn Val Thr Asn Ser Thr Glu Ala Ala Ser Ala Ser  
 515 520 525  
 Ala Ala Ser Ala Ser Gln Ser Ser Ala Ala Ala Thr Gln Thr Asp  
 530 535 540  
 Val Ser Ser Thr Thr Thr Ser Tyr Leu Arg Thr Thr Pro Thr Pro Gly  
 545 550 555 560  
 Val Arg Asp Ile Asn Tyr Pro Pro Tyr Val Ile Asn His Val Gln Ser  
 565 570 575  
 Gly His Asp Leu Ala Val His Ala Ile Ser Pro Asn Ala Thr His Val  
 580 585 590  
 Asp Gly Val Gln Glu Tyr Asp Val His Ser Leu Trp Gly His Gln Ile  
 595 600 605  
 Leu Asn Ala Thr Tyr Gln Gly Leu Leu Glu Val Phe Thr Glu Lys Arg  
 610 615 620  
 Pro Phe Ile Ile Gly Arg Ser Thr Phe Ala Gly Ser Gly Lys Trp Ala  
 625 630 635 640  
 Gly His Trp Gly Gly Asp Asn Asn Ser Arg Trp Gly Ser Met Phe His  
 645 650 655  
 Ser Ile Ser Gln Ala Leu Ser Phe Ser Leu Phe Gly Ile Pro Met Phe  
 660 665 670  
 Gly Val Asp Thr Cys Gly Phe Asn Gly Asn Thr Asp Glu Glu Leu Cys  
 675 680 685  
 Asn Arg Trp Met Gln Leu Ser Ala Phe Phe Pro Phe Tyr Arg Asn His  
 690 695 700

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Asn Thr Leu Ala Ala Leu Ser Gln Glu Pro Tyr Arg Trp Ala Ser Val  
 705 710 715 720  
 Thr Glu Ala Ala Lys Thr Ala Met Ser Ile Arg Tyr Ala Leu Leu Pro  
 725 730 735  
 Tyr Phe Tyr Thr Leu Phe His Gln Ala His Thr Thr Gly Ser Thr Val  
 740 745 750  
 Met Arg Ala Leu Ala Trp Glu Phe Pro Asn Asp Pro Ser Leu Ala Ala  
 755 760 765  
 Val Asp Thr Gln Phe Met Val Gly Pro Ser Ile Leu Val Thr Pro Val  
 770 775 780  
 Leu Glu Pro Leu Ala Lys Thr Val Lys Gly Val Phe Pro Gly Val Gly  
 785 790 795 800  
 Lys Gly Gln Val Trp Tyr Asp Trp Tyr Thr Gln Ala Ala Val Asp Ala  
 805 810 815  
 Lys Pro Gly Val Asn Thr Thr Ile Pro Ala Pro Leu Gly His Ile Pro  
 820 825 830  
 Val Tyr Val Arg Gly Gly Ser Ile Leu Pro Met Gln Glu Pro Ala Leu  
 835 840 845  
 Thr Thr Arg Asp Ala Arg Lys Thr Pro Trp Ser Leu Leu Ala Ala Leu  
 850 855 860  
 Asp Gly Asn Gln Thr Ala Ser Gly Gln Leu Tyr Leu Asp Asp Gly Ser  
 865 870 875 880  
 Ser Val Asn Pro Ser Ser Thr Leu Asn Val Glu Phe Ala Ala Thr His  
 885 890 895  
 Ser Ser Ile Lys Val Ser Ala Lys Gly Asp Trp Arg Glu Lys Asn Ser  
 900 905 910  
 Leu Asp Ser Val Thr Val Leu Gly Val Ala Lys Glu Pro Ala Arg Val  
 915 920 925  
 Thr Phe Asn Arg Arg Arg Val Pro Pro Glu Ser Val Glu Tyr Asn Ala  
 930 935 940  
 Thr Ser Gln Val Leu Thr Val Ser Gly Leu Gln Lys Leu Thr Pro Arg  
 945 950 955 960  
 Gly Ala Trp Ala Glu Asp Trp Ile Leu Lys Trp  
 965 970

&lt;210&gt; SEQ ID NO 7

&lt;211&gt; LENGTH: 3158

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Neosartorya fischeri

&lt;400&gt; SEQUENCE: 7

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 agccattcgc ttgcgctag cacgtccgca acctcagcac acgcgcaata cactttacca 120  
 tcttctattg acgttggtgc tcaattggtc gccaacatcg acgatccct tgccgtcgac 180  
 gcacagtctg tgtgtccggg ctacaaagcc tcaaatgtgc accagacatc ccaaggtttc 240  
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 tacgtggatg cctccaacgc ttcttggtag ctctctcgg aagacttggg gccccgggct 420  
 caaggctctg gcgtgtccgc ctctcaaagc gactttgatg tgaagtggtc caatgagcct 480  
 tctttcaacc tcaaggtcat tcgcaaggct actggagacg tcctcttcga taccgagggc 540  
 tccgtcctgg tctttgagaa ccagtttata gagtttgtct ctctcgttgc cgagggttac 600

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aacctgtaag gtttgggaga ggcgatggcc cagctgcggc tcttgagaaa cgcgaccctg	660
accacctatg cagcggatgt gggagaccgg attgataggt atgttgctga ccatggttga	720
aacctaatgt acgaagtoga caagcttaca atcggctctc cagcaacatc tatggacagc	780
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tcaacacgga cgaggcggac ttgtcggagg attatgaatc attctccac ggtgtctttc	900
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gctaccagct ctccaccatt ggacttctct caatgcagca gtacagcacc cttggattcc	1080
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tggtcgtccc cgtgctcgag cccctcggc ataccgtcaa gggcgcgttc ccaggcgtcg	2640
gcaaaggcga agtctggtac gactggtaca cccagaccgc cgtagacgcc aaacccggcg	2700
tcaacaccac cattcccgtc ccgctgggac acattcccgt ctatgtcgt ggaggcagca	2760
tcctgcccac gcaggaaccc gccctcacga ccagagacgc ccgtaaacct ccctggtcgc	2820
tactcgtcgc tctgagcggc aaccagactg cctcgggctc gctgtatctc gaecagcga	2880
ccagcctcaa cccgtcccgc actctcgtg tcgacttcca ggctaccgcc tggagcatca	2940
aggtctcggg caagggtacc tgggaggaga agaaccgctt ggataagggt actgtcctcg	3000

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gcgtgggtga gaagccttcc gctgtgacgt tcaacggccg caacgtccac cctggctcgg 3060
tgcactacaa tgctacctcc aaggtgctgt ctgtgcaggg attgcacagc atgacgcccc 3120
atggcgcctg ggctggaaac tgggttctga aatggtag 3158

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<210> SEQ ID NO 8
<211> LENGTH: 988
<212> TYPE: PRT
<213> ORGANISM: Neosartorya fischeri

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<400> SEQUENCE: 8

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Met Ala Ser Leu Leu Gly Leu Val Ala Ser Ala Trp Leu Leu Pro Thr
1          5          10          15
Ala Tyr Gly Ala Ser His Ser Leu Ala Pro Ser Thr Ser Ala Thr Ser
20          25          30
Ala His Ala Gln Tyr Thr Leu Pro Ser Ser Ile Asp Val Gly Ala Gln
35          40          45
Leu Val Ala Asn Ile Asp Asp Pro Leu Ala Val Asp Ala Gln Ser Val
50          55          60
Cys Pro Gly Tyr Lys Ala Ser Asn Val His Gln Thr Ser Gln Gly Phe
65          70          75          80
Thr Ala Ser Leu Gln Leu Ala Gly Asp Pro Cys Asn Val Tyr Gly Thr
85          90          95
Asp Val Asp Ser Leu Ser Leu Thr Val Asp Tyr Leu Ala Lys Asp Arg
100         105         110
Leu Asn Ile Gln Ile Val Pro Thr Tyr Val Asp Ala Ser Asn Ala Ser
115         120         125
Trp Tyr Leu Leu Ser Glu Asp Leu Val Pro Arg Ala Gln Gly Ser Gly
130         135         140
Val Ser Ala Ser Gln Ser Asp Phe Asp Val Lys Trp Ser Asn Glu Pro
145         150         155         160
Ser Phe Asn Leu Lys Val Ile Arg Lys Ala Thr Gly Asp Val Leu Phe
165         170         175
Asp Thr Glu Gly Ser Val Leu Val Phe Glu Asn Gln Phe Ile Glu Phe
180         185         190
Val Ser Ser Leu Pro Glu Gly Tyr Asn Leu Tyr Gly Leu Gly Glu Arg
195         200         205
Met Ala Gln Leu Arg Leu Leu Arg Asn Ala Thr Leu Thr Thr Tyr Ala
210         215         220
Ala Asp Val Gly Asp Pro Ile Asp Ser Asn Ile Tyr Gly Gln His Pro
225         230         235         240
Phe Tyr Leu Asp Thr Arg Tyr Tyr Thr Lys Gly Thr Asn Gly Ser Tyr
245         250         255
Ser Leu Val Asn Thr Asp Glu Ala Asp Leu Ser Glu Asp Tyr Glu Ser
260         265         270
Phe Ser His Gly Val Phe Leu Arg Asn Ser His Gly Gln Glu Val Leu
275         280         285
Leu Gln Pro Arg Asn Ile Thr Trp Arg Thr Ile Gly Gly Ser Ile Asp
290         295         300
Leu Thr Phe Tyr Ser Gly Pro Thr Gln Ala Asp Val Thr Lys Ser Tyr
305         310         315         320
Gln Leu Ser Thr Ile Gly Leu Pro Ala Met Gln Gln Tyr Ser Thr Leu
325         330         335
Gly Phe His Gln Cys Arg Trp Gly Tyr Gln Asn Trp Ser Gln Leu Glu

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340					345					350					
Glu	Val	Val	Asn	Asn	Phe	Glu	Arg	Phe	Glu	Ile	Pro	Leu	Glu	Tyr	Ile
		355					360					365			
Trp	Ser	Asp	Ile	Asp	Tyr	Met	Leu	Gly	Tyr	Arg	Asp	Phe	Glu	Asn	Asp
	370					375					380				
Pro	Glu	Arg	Phe	Ser	Tyr	Asp	Glu	Gly	Glu	Glu	Phe	Leu	Asn	Lys	Leu
	385				390					395					400
His	Lys	Ser	Gly	Arg	His	Tyr	Val	Pro	Ile	Val	Asp	Ser	Ala	Ile	Tyr
				405					410						415
Ile	Pro	Asn	Pro	Asp	Asn	Ala	Ser	Asp	Ala	Tyr	Glu	Pro	Tyr	Ala	Arg
		420						425					430		
Gly	Ala	Lys	Asp	Asp	Val	Phe	Ile	Lys	Asn	Pro	Asp	Gly	Thr	Leu	Tyr
		435					440					445			
Ile	Gly	Ala	Val	Trp	Pro	Gly	Phe	Thr	Val	Phe	Pro	Asp	Trp	Leu	Asn
	450					455					460				
Pro	Lys	Ala	Phe	Asp	Tyr	Trp	Ala	Asn	Glu	Leu	Val	Ile	Trp	Ser	Lys
	465				470					475					480
Lys	Val	Ala	Phe	Asp	Gly	Ile	Trp	Ile	Asp	Met	Ser	Glu	Val	Ser	Ser
			485						490						495
Phe	Cys	Val	Gly	Ser	Cys	Gly	Thr	Gly	Lys	Leu	His	Leu	Asn	Pro	Val
		500						505						510	
His	Pro	Pro	Phe	Gln	Leu	Pro	Gly	Glu	Pro	Gly	Asn	Ile	Gly	Tyr	Asp
		515					520						525		
Tyr	Pro	Glu	Ala	Phe	Asn	Val	Thr	Asn	Ser	Thr	Glu	Ala	Ala	Ser	Ala
	530					535					540				
Ser	Ala	Ala	Ser	Ala	Ser	Gln	Ala	Ser	Ala	Ala	Ala	Ala	Thr	Gln	Thr
	545				550					555					560
Ala	Thr	Thr	Ser	Thr	Ser	Thr	Ser	Tyr	Leu	Arg	Thr	Thr	Pro	Thr	Pro
				565					570						575
Gly	Val	Arg	Asp	Val	Asn	Tyr	Pro	Pro	Tyr	Val	Ile	Asn	His	Val	Gln
		580						585						590	
Glu	Gly	His	Asp	Leu	Ala	Val	His	Ala	Ile	Ser	Pro	Asn	Ser	Thr	His
		595					600						605		
Ala	Asp	Gly	Val	Gln	Glu	Tyr	Asp	Val	His	Ser	Leu	Trp	Gly	His	Gln
	610					615					620				
Ile	Leu	Asn	Ala	Thr	Tyr	Tyr	Gly	Leu	Arg	Gln	Val	Phe	Thr	Glu	Lys
	625				630					635					640
Arg	Pro	Phe	Ile	Ile	Gly	Arg	Ser	Thr	Phe	Ala	Gly	Ser	Gly	Lys	Trp
				645					650						655
Ala	Gly	His	Trp	Gly	Gly	Asp	Asn	Asn	Ser	Lys	Trp	Gly	Ser	Met	Phe
			660					665						670	
Leu	Ser	Ile	Ser	Gln	Gly	Leu	Ser	Phe	Ser	Leu	Phe	Gly	Ile	Pro	Met
		675					680						685		
Phe	Gly	Val	Asp	Thr	Cys	Gly	Phe	Asn	Gly	Asn	Thr	Asp	Glu	Glu	Leu
	690					695						700			
Cys	Ser	Arg	Trp	Met	Gln	Leu	Ser	Ala	Phe	Phe	Pro	Phe	Tyr	Arg	Asn
	705				710					715					720
His	Asn	Val	Leu	Gly	Ala	Ile	Pro	Gln	Glu	Pro	Tyr	Arg	Trp	Ala	Ser
				725						730					735
Val	Thr	Gln	Ala	Ser	Lys	Ala	Ala	Met	Lys	Ile	Arg	Tyr	Ser	Ile	Leu
			740						745					750	
Pro	Tyr	Phe	Tyr	Thr	Leu	Phe	His	Gln	Ala	His	Thr	Thr	Gly	Ser	Thr
		755						760							765

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Val Met Arg Ala Leu Ala Trp Glu Phe Pro Thr Asp Pro Ser Leu Ala  
 770 775 780  
 Ala Val Asp Thr Gln Phe Met Val Gly Pro Ser Ile Met Val Val Pro  
 785 790 795 800  
 Val Leu Glu Pro Leu Ala Asp Thr Val Lys Gly Ala Phe Pro Gly Val  
 805 810 815  
 Gly Lys Gly Glu Val Trp Tyr Asp Trp Tyr Thr Gln Thr Ala Val Asp  
 820 825 830  
 Ala Lys Pro Gly Val Asn Thr Thr Ile Pro Ala Pro Leu Gly His Ile  
 835 840 845  
 Pro Val Tyr Val Arg Gly Gly Ser Ile Leu Pro Met Gln Glu Pro Ala  
 850 855 860  
 Leu Thr Thr Arg Asp Ala Arg Asn Thr Pro Trp Ser Leu Leu Val Ala  
 865 870 875 880  
 Leu Ser Gly Asn Gln Thr Ala Ser Gly Ser Leu Tyr Leu Asp Asp Gly  
 885 890 895  
 Thr Ser Leu Asn Pro Ser Arg Thr Leu Asp Val Asp Phe Gln Ala Thr  
 900 905 910  
 Ala Trp Ser Ile Lys Val Ser Val Lys Gly Thr Trp Glu Glu Lys Asn  
 915 920 925  
 Arg Leu Asp Lys Val Thr Val Leu Gly Val Gly Glu Lys Pro Ser Ala  
 930 935 940  
 Val Thr Phe Asn Gly Arg Asn Val His Pro Gly Ser Val His Tyr Asn  
 945 950 955 960  
 Ala Thr Ser Lys Val Leu Ser Val Gln Gly Leu His Ser Met Thr Pro  
 965 970 975  
 His Gly Ala Trp Ala Gly Asn Trp Val Leu Lys Trp  
 980 985

&lt;210&gt; SEQ ID NO 9

&lt;211&gt; LENGTH: 969

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Neosartorya fischeri*

&lt;400&gt; SEQUENCE: 9

Ala Ser His Ser Leu Ala Pro Ser Thr Ser Ala Thr Ser Ala His Ala  
 1 5 10 15  
 Gln Tyr Thr Leu Pro Ser Ser Ile Asp Val Gly Ala Gln Leu Val Ala  
 20 25 30  
 Asn Ile Asp Asp Pro Leu Ala Val Asp Ala Gln Ser Val Cys Pro Gly  
 35 40 45  
 Tyr Lys Ala Ser Asn Val His Gln Thr Ser Gln Gly Phe Thr Ala Ser  
 50 55 60  
 Leu Gln Leu Ala Gly Asp Pro Cys Asn Val Tyr Gly Thr Asp Val Asp  
 65 70 75 80  
 Ser Leu Ser Leu Thr Val Asp Tyr Leu Ala Lys Asp Arg Leu Asn Ile  
 85 90 95  
 Gln Ile Val Pro Thr Tyr Val Asp Ala Ser Asn Ala Ser Trp Tyr Leu  
 100 105 110  
 Leu Ser Glu Asp Leu Val Pro Arg Ala Gln Gly Ser Gly Val Ser Ala  
 115 120 125  
 Ser Gln Ser Asp Phe Asp Val Lys Trp Ser Asn Glu Pro Ser Phe Asn  
 130 135 140  
 Leu Lys Val Ile Arg Lys Ala Thr Gly Asp Val Leu Phe Asp Thr Glu



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Asp Leu Ala Val His Ala Ile Ser Pro Asn Ser Thr His Ala Asp Gly  
                   580                                  585                                  590

Val Gln Glu Tyr Asp Val His Ser Leu Trp Gly His Gln Ile Leu Asn  
                   595                                  600                                  605

Ala Thr Tyr Tyr Gly Leu Arg Gln Val Phe Thr Glu Lys Arg Pro Phe  
                   610                                  615                                  620

Ile Ile Gly Arg Ser Thr Phe Ala Gly Ser Gly Lys Trp Ala Gly His  
                   625                                  630                                  635                                  640

Trp Gly Gly Asp Asn Asn Ser Lys Trp Gly Ser Met Phe Leu Ser Ile  
                                   645                                  650                                  655

Ser Gln Gly Leu Ser Phe Ser Leu Phe Gly Ile Pro Met Phe Gly Val  
                   660                                  665                                  670

Asp Thr Cys Gly Phe Asn Gly Asn Thr Asp Glu Glu Leu Cys Ser Arg  
                   675                                  680                                  685

Trp Met Gln Leu Ser Ala Phe Phe Pro Phe Tyr Arg Asn His Asn Val  
                   690                                  695                                  700

Leu Gly Ala Ile Pro Gln Glu Pro Tyr Arg Trp Ala Ser Val Thr Gln  
                   705                                  710                                  715                                  720

Ala Ser Lys Ala Ala Met Lys Ile Arg Tyr Ser Ile Leu Pro Tyr Phe  
                                   725                                  730                                  735

Tyr Thr Leu Phe His Gln Ala His Thr Thr Gly Ser Thr Val Met Arg  
                   740                                  745                                  750

Ala Leu Ala Trp Glu Phe Pro Thr Asp Pro Ser Leu Ala Ala Val Asp  
                   755                                  760                                  765

Thr Gln Phe Met Val Gly Pro Ser Ile Met Val Val Pro Val Leu Glu  
                   770                                  775                                  780

Pro Leu Ala Asp Thr Val Lys Gly Ala Phe Pro Gly Val Gly Lys Gly  
                   785                                  790                                  795                                  800

Glu Val Trp Tyr Asp Trp Tyr Thr Gln Thr Ala Val Asp Ala Lys Pro  
                                   805                                  810                                  815

Gly Val Asn Thr Thr Ile Pro Ala Pro Leu Gly His Ile Pro Val Tyr  
                                   820                                  825                                  830

Val Arg Gly Gly Ser Ile Leu Pro Met Gln Glu Pro Ala Leu Thr Thr  
                   835                                  840                                  845

Arg Asp Ala Arg Asn Thr Pro Trp Ser Leu Leu Val Ala Leu Ser Gly  
                   850                                  855                                  860

Asn Gln Thr Ala Ser Gly Ser Leu Tyr Leu Asp Asp Gly Thr Ser Leu  
                   865                                  870                                  875                                  880

Asn Pro Ser Arg Thr Leu Asp Val Asp Phe Gln Ala Thr Ala Trp Ser  
                                   885                                  890                                  895

Ile Lys Val Ser Val Lys Gly Thr Trp Glu Glu Lys Asn Arg Leu Asp  
                   900                                  905                                  910

Lys Val Thr Val Leu Gly Val Gly Glu Lys Pro Ser Ala Val Thr Phe  
                   915                                  920                                  925

Asn Gly Arg Asn Val His Pro Gly Ser Val His Tyr Asn Ala Thr Ser  
                   930                                  935                                  940

Lys Val Leu Ser Val Gln Gly Leu His Ser Met Thr Pro His Gly Ala  
                   945                                  950                                  955                                  960

Trp Ala Gly Asn Trp Val Leu Lys Trp  
                   965

&lt;210&gt; SEQ ID NO 10

&lt;211&gt; LENGTH: 3385

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&lt;212&gt; TYPE: DNA

<213> ORGANISM: *Neurospora crassa*

&lt;400&gt; SEQUENCE: 10

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atggccgtct tcacatcggc ttcttcttt cttcttgcgg ctcttgtecc tcaatggaca    60
tctgctcaac atgtatctgt ggttgcaacg tcactctggac ctggtgtgtt gagcgggacg    120
gtggcagggg attcccctat gttcactttc ccagcttcgg ctgacatcgg accaaatgtc    180
ttaccgaaca ttttgcagcc gcaagcagtt aatgttcaaa gcgtctgtcc aggatataca    240
gctgctaata cacaaaagac ggagaaggga ctcacggctg acttgaccct tgctggccct    300
ccctgcaatg tctatggcaa cgacatcgag cacctgaagc ttaccattga gtttcaggcg    360
gacaatcgga tcaatgtcca gattcaacct cgctatactg gccccggtaa tgaaacctgg    420
ttcatacttc ctgaggtgct cgtgccacga ccagaggccg agcctgatgc gaatgccgct    480
agaagcaagc tggaaatctc gtggtcgaat gagcccacct tctccttcac agtgaagcgt    540
aaggagactg gagatgtctt gttcacgacc gagggccgtg tccttgttta tgaggatcag    600
ttcatcgagt tcggctcttc tttaccggag aattacaacc tgatatgtct cggcgaagtt    660
atgcatggct ttagactggg gaacaatctg acacgtaagt ctctttactt aacatgaatg    720
tgcacgcgtg attgcatctg actaacacca tctccagcca cgctgttcgc tgcggatgtg    780
ggcgacaacc tcgatgcca catctacggc aaccacccga tctatctoga caccagatac    840
ttcaccaagg acgagtctgg aaaattaagc tacgtttccg acccagcaga caagaatgcc    900
aaatatgttt cgtatacaaa cgtgtctctc cttcgaaatg ctcatgcaca ggaagtgtc    960
cttcgaccgg aaggtatcac ctggaggact ctcggcggaa gcacgattt gtacttcttt   1020
gagggacctt ttgctcagga tatcatcaag tcttatcagc tcagtaccgt tggctcttct   1080
gcaatgcagc agtattggac tcttgcttt caccaatgtc gctggggata ctcgaactgg   1140
actgttgtag aggatgtctg tgacaatttc cgcaagtgtg gcaccccggt ggagacaatc   1200
tggagtgagt gaacccaagt ccttcagcat cagaatcttc cccatccatg cttttactga   1260
cttttttaag ccgacattga ttacatgaag ggctatcgtg actttgaaaa cgaccctgat   1320
cagttcagct acgaggaagg cgccagggtc ctcgaggagc ttcacaagaa tcaccagcac   1380
tacgtgccga ttgtcgactc ggccatctat gttccaaatc ctgacaagcc agaagatgat   1440
tacgaacctt accaccgtgg actcgaagct gatgctttca tcatgaacct agacggttcg   1500
ctctatatcg gtgcagtgtg gctcggctac accgtgttcc cggactggat tggtgccgcc   1560
ctcaacggta caggtaccgt cgctgggtgg acggacgagt ttgtaggta ctataagaag   1620
gtcgtctttg atggcatctg gattgacatg agcgagggtg cttctttctg catcgggaagc   1680
tgtggcacgg gcaacctgac gctcaatccg gttcatccac catgggggtc tcccggtgag   1740
ccaggcgctc ttgtgctcga ttacccgaa ggttttgaaa aaaccaacgc gactgaggca   1800
tcctctgcaa cgctcggtta caaaacgcag aaccagacc ccacgactac agccagcacc   1860
actagcacta cttcttaoct caggacgaca ccgactcctg gactgcgcaa tatcaactat   1920
ccaccatatt tcatcaacaa cttccacggc gacattggta ctcacgcctt gagccccaat   1980
ggtacccaac atggcggcac agtcgactat gacttcaca acttgctcgg ccatcagatc   2040
ctccacgcaa cctaccaggc acttctcaaa gtctttgagg gcaagcgtcc ttttateatc   2100
ggccgcagca cctttgctgg ttctggcaaa tgggcgggtc actggggtgg tgataactat   2160
tccctatggg cgtttctgta ctttagcatt cccaggeccc taccctctc catcttcggt   2220

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ttccccatgt tcggcgtaga tacctgcggc ttcaatggca acacggacca cgagctgtgc 2280
tcacgatgga tgcagctcag cgccttttcc ccattctacc gcaaccacaa cgtccgtggc 2340
gccatcagcc aggagcccta cgtgtgggagc tctgtgatcg atgcgtccaa gaaggcgatg 2400
aggattcgat acctcctgct cccgtacatg tacacactta tggcccaggc cagcctgtct 2460
ggagatacgg tcattgcgcg actctcgtgg gagttcccgc aagagccgtg gttggcggat 2520
gcggatcgcc agttcatggt gggcagcgcg gtgatggta caccatgtct tgttcaagg 2580
gccaatacag tggacggcgt gtttctgga gttggcgatg ggaccatctg gtatgattgg 2640
tatacataca aggccgcag tgagggtgtt cagcctgggg agaatgtaac gatcgatgca 2700
cctctgggac acattccggt ttttctacgg gttggccatg tcattccagt gcaagagccg 2760
ggcatgacta cgacggagag cagacaaaat gaggggagtg tcatcgttgc tcttgatggt 2820
gcggttaagg cgaatggtac gttgtatctg gatgatggtg agagtttga gccgggtgag 2880
aatgtgaagt gggttgatgt gagtcttctt ccgttcttcc tatttttctt ctcttttccg 2940
tgagtgttca gtcggtcgat tgccactcgc tcctctcgcg acaaggtcga aagtgctaac 3000
cggttttggc tgtttctagt tcacggttga gaagaactca tttcgagtga cacctcaggg 3060
caagtacctt gaccgaaact cactggccaa cgtcaccgac ctggggagtgg ccgaggcacc 3120
tctgggagtg gctattaata gtcactcgtc cggatcagct tcttggctct acgactccga 3180
ggggaagtcc ctttcggtaa ccgagctgca ggacaacttc aaggaagggg cgtgggcatc 3240
caactggaag ctgtcgtgga actcggcctc aaactcgggc tcgtctcctg ttcagggagg 3300
cggcggcagg ctcgagttca gcacgcccac tttgctccat gcagctgctt tcggcatcct 3360
ttttggccgc atgtttgtag ttttag 3385

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&lt;210&gt; SEQ ID NO 11

&lt;211&gt; LENGTH: 1044

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Neurospora crassa

&lt;400&gt; SEQUENCE: 11

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Met Ala Val Phe Thr Ser Ala Ser Phe Phe Leu Leu Ala Ala Leu Val
1             5             10            15

Pro Gln Trp Thr Ser Ala Gln His Val Ser Val Val Ala Thr Ser Ser
20           25           30

Gly Pro Gly Val Leu Ser Gly Thr Val Ala Gly Asp Ser Pro Met Phe
35           40           45

Thr Phe Pro Ala Ser Ala Asp Ile Gly Pro Asn Val Leu Pro Asn Ile
50           55           60

Phe Asp Pro Gln Ala Val Asn Val Gln Ser Val Cys Pro Gly Tyr Thr
65           70           75           80

Ala Ala Asn Ala Gln Lys Thr Glu Lys Gly Leu Thr Ala Asp Leu Thr
85           90           95

Leu Ala Gly Pro Pro Cys Asn Val Tyr Gly Asn Asp Ile Glu His Leu
100          105          110

Lys Leu Thr Ile Glu Phe Gln Ala Asp Asn Arg Ile Asn Val Gln Ile
115          120          125

Gln Pro Arg Tyr Thr Gly Pro Gly Asn Glu Thr Trp Phe Ile Leu Pro
130          135          140

Glu Val Leu Val Pro Arg Pro Glu Ala Glu Pro Asp Ala Asn Ala Ala
145          150          155          160

Arg Ser Lys Leu Glu Ile Ser Trp Ser Asn Glu Pro Thr Phe Ser Phe

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165				170				175							
Thr	Val	Lys	Arg	Lys	Glu	Thr	Gly	Asp	Val	Leu	Phe	Thr	Thr	Glu	Gly
			180								185				190
Arg	Val	Leu	Val	Tyr	Glu	Asp	Gln	Phe	Ile	Glu	Phe	Gly	Ser	Ser	Leu
		195					200								205
Pro	Glu	Asn	Tyr	Asn	Leu	Tyr	Gly	Leu	Gly	Glu	Val	Met	His	Gly	Phe
		210				215					220				
Arg	Leu	Gly	Asn	Asn	Leu	Thr	Arg	Thr	Leu	Phe	Ala	Ala	Asp	Val	Gly
		225				230					235				240
Asp	Asn	Leu	Asp	Ala	Asn	Ile	Tyr	Gly	Asn	His	Pro	Ile	Tyr	Leu	Asp
					245					250					255
Thr	Arg	Tyr	Phe	Thr	Lys	Asp	Glu	Ser	Gly	Lys	Leu	Ser	Tyr	Val	Ser
			260							265					270
Asp	Pro	Ala	Asp	Lys	Asn	Ala	Lys	Tyr	Val	Ser	Tyr	Thr	Asn	Gly	Val
		275					280								285
Phe	Leu	Arg	Asn	Ala	His	Ala	Gln	Glu	Val	Leu	Leu	Arg	Pro	Glu	Gly
		290				295									300
Ile	Thr	Trp	Arg	Thr	Leu	Gly	Gly	Ser	Ile	Asp	Leu	Tyr	Phe	Phe	Glu
		305				310					315				320
Gly	Pro	Phe	Ala	Gln	Asp	Ile	Ile	Lys	Ser	Tyr	Gln	Leu	Ser	Thr	Val
					325						330				335
Gly	Leu	Pro	Ala	Met	Gln	Gln	Tyr	Trp	Thr	Leu	Gly	Phe	His	Gln	Cys
			340								345				350
Arg	Trp	Gly	Tyr	Ser	Asn	Trp	Thr	Val	Val	Lys	Asp	Val	Val	Asp	Asn
		355					360								365
Phe	Arg	Lys	Phe	Gly	Ile	Pro	Leu	Glu	Thr	Ile	Trp	Thr	Asp	Ile	Asp
		370				375					380				
Tyr	Met	Lys	Gly	Tyr	Arg	Asp	Phe	Glu	Asn	Asp	Pro	Asp	Gln	Phe	Ser
		385				390					395				400
Tyr	Glu	Glu	Gly	Ala	Arg	Phe	Leu	Glu	Glu	Leu	His	Lys	Asn	His	Gln
					405						410				415
His	Tyr	Val	Pro	Ile	Val	Asp	Ser	Ala	Ile	Tyr	Val	Pro	Asn	Pro	Asp
			420												430
Lys	Pro	Glu	Asp	Asp	Tyr	Glu	Pro	Tyr	His	Arg	Gly	Leu	Glu	Ala	Asp
		435					440								445
Ala	Phe	Ile	Met	Asn	Pro	Asp	Gly	Ser	Leu	Tyr	Ile	Gly	Ala	Val	Trp
		450				455					460				
Pro	Gly	Tyr	Thr	Val	Phe	Pro	Asp	Trp	Ile	Gly	Ala	Ala	Leu	Asn	Gly
		465				470					475				480
Thr	Gly	Thr	Val	Gly	Trp	Trp	Thr	Asp	Glu	Phe	Val	Arg	Tyr	Tyr	Lys
					485						490				495
Lys	Val	Ala	Phe	Asp	Gly	Ile	Trp	Ile	Asp	Met	Ser	Glu	Val	Ala	Ser
			500								505				510
Phe	Cys	Ile	Gly	Ser	Cys	Gly	Thr	Gly	Asn	Leu	Thr	Leu	Asn	Pro	Val
		515					520								525
His	Pro	Pro	Trp	Gly	Leu	Pro	Gly	Glu	Pro	Gly	Ala	Leu	Val	Leu	Asp
		530				535					540				
Tyr	Pro	Glu	Gly	Phe	Glu	Lys	Thr	Asn	Ala	Ser	Glu	Ala	Ser	Ser	Ala
		545				550					555				560
Thr	Ser	Val	Tyr	Lys	Thr	Gln	Asn	Pro	Asp	Pro	Thr	Thr	Thr	Ala	Ser
					565						570				575
Thr	Thr	Ser	Thr	Thr	Ser	Tyr	Leu	Arg	Thr	Thr	Pro	Thr	Pro	Gly	Val
			580								585				590

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Arg Asn Ile Asn Tyr Pro Pro Tyr Val Ile Asn Asn Phe His Gly Asp  
           595                                  600                                  605

Ile Gly Thr His Ala Leu Ser Pro Asn Gly Thr His His Gly Gly Thr  
       610                                  615                                  620

Val Asp Tyr Asp Phe His Asn Leu Phe Gly His Gln Ile Leu His Ala  
       625                                  630                                  635                                  640

Thr Tyr Gln Ala Leu Leu Lys Val Phe Glu Gly Lys Arg Pro Phe Ile  
                                   645                                  650                                  655

Ile Gly Arg Ser Thr Phe Ala Gly Ser Gly Lys Trp Ala Gly His Trp  
                                   660                                  665                                  670

Gly Gly Asp Asn Tyr Ser Leu Trp Ala Phe Leu Tyr Phe Ser Ile Pro  
                                   675                                  680                                  685

Gln Ala Leu Ser Phe Ser Ile Phe Gly Phe Pro Met Phe Gly Val Asp  
       690                                  695                                  700

Thr Cys Gly Phe Asn Gly Asn Thr Asp His Glu Leu Cys Ser Arg Trp  
       705                                  710                                  715                                  720

Met Gln Leu Ser Ala Phe Phe Pro Phe Tyr Arg Asn His Asn Val Arg  
                                   725                                  730                                  735

Gly Ala Ile Ser Gln Glu Pro Tyr Val Trp Ser Ser Val Ile Asp Ala  
                                   740                                  745                                  750

Ser Lys Lys Ala Met Arg Ile Arg Tyr Leu Leu Leu Pro Tyr Met Tyr  
                                   755                                  760                                  765

Thr Leu Met Ala Gln Ala Ser Leu Ser Gly Asp Thr Val Met Arg Ala  
       770                                  775                                  780

Leu Ser Trp Glu Phe Pro Gln Glu Pro Trp Leu Ala Asp Ala Asp Arg  
       785                                  790                                  795                                  800

Gln Phe Met Leu Gly Ser Ala Val Met Val Thr Pro Cys Leu Val Gln  
                                   805                                  810                                  815

Gly Ala Asn Thr Val Asp Gly Val Phe Pro Gly Val Gly Asp Gly Thr  
                                   820                                  825                                  830

Ile Trp Tyr Asp Trp Tyr Thr Tyr Lys Ala Ala Ser Glu Gly Val Gln  
                                   835                                  840                                  845

Pro Gly Glu Asn Val Thr Ile Asp Ala Pro Leu Gly His Ile Pro Val  
       850                                  855                                  860

Phe Leu Arg Gly Gly His Val Ile Pro Val Gln Glu Pro Gly Met Thr  
       865                                  870                                  875                                  880

Thr Thr Glu Ser Arg Gln Asn Glu Trp Ser Val Ile Val Ala Leu Asp  
                                   885                                  890                                  895

Gly Ala Gly Lys Ala Asn Gly Thr Leu Tyr Leu Asp Asp Gly Glu Ser  
                                   900                                  905                                  910

Leu Glu Pro Gly Glu Asn Val Lys Trp Val Asp Phe Thr Val Glu Lys  
       915                                  920                                  925

Asn Ser Phe Arg Val Thr Pro Gln Gly Lys Tyr Leu Asp Arg Asn Ser  
       930                                  935                                  940

Leu Ala Asn Val Thr Ile Leu Gly Val Ala Glu Ala Pro Leu Gly Val  
       945                                  950                                  955                                  960

Ala Ile Asn Ser His Leu Leu Gly Ser Ala Ser Trp Ser Tyr Asp Ser  
                                   965                                  970                                  975

Glu Gly Lys Phe Leu Ser Val Thr Glu Leu Gln Asp Asn Phe Lys Glu  
                                   980                                  985                                  990

Gly Ala Trp Ala Ser Asn Trp Thr Leu Ser Trp Asn Ser Ala Ser Asn  
       995                                  1000                                  1005

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Ser Gly Ser Ser Pro Val Gln Gly Gly Gly Gly Arg Leu Glu Phe  
1010 1015 1020

Ser Thr Pro Asn Leu Leu His Ala Ala Ala Phe Gly Ile Leu Phe  
1025 1030 1035

Gly Arg Met Phe Val Val  
1040

<210> SEQ ID NO 12

<211> LENGTH: 1022

<212> TYPE: PRT

<213> ORGANISM: *Neurospora crassa*

<400> SEQUENCE: 12

Gln His Val Ser Val Val Ala Thr Ser Ser Gly Pro Gly Val Leu Ser  
1 5 10 15

Gly Thr Val Ala Gly Asp Ser Pro Met Phe Thr Phe Pro Ala Ser Ala  
20 25 30

Asp Ile Gly Pro Asn Val Leu Pro Asn Ile Phe Asp Pro Gln Ala Val  
35 40 45

Asn Val Gln Ser Val Cys Pro Gly Tyr Thr Ala Ala Asn Ala Gln Lys  
50 55 60

Thr Glu Lys Gly Leu Thr Ala Asp Leu Thr Leu Ala Gly Pro Pro Cys  
65 70 75 80

Asn Val Tyr Gly Asn Asp Ile Glu His Leu Lys Leu Thr Ile Glu Phe  
85 90 95

Gln Ala Asp Asn Arg Ile Asn Val Gln Ile Gln Pro Arg Tyr Thr Gly  
100 105 110

Pro Gly Asn Glu Thr Trp Phe Ile Leu Pro Glu Val Leu Val Pro Arg  
115 120 125

Pro Glu Ala Glu Pro Asp Ala Asn Ala Ala Arg Ser Lys Leu Glu Ile  
130 135 140

Ser Trp Ser Asn Glu Pro Thr Phe Ser Phe Thr Val Lys Arg Lys Glu  
145 150 155 160

Thr Gly Asp Val Leu Phe Thr Thr Glu Gly Arg Val Leu Val Tyr Glu  
165 170 175

Asp Gln Phe Ile Glu Phe Gly Ser Ser Leu Pro Glu Asn Tyr Asn Leu  
180 185 190

Tyr Gly Leu Gly Glu Val Met His Gly Phe Arg Leu Gly Asn Asn Leu  
195 200 205

Thr Arg Thr Leu Phe Ala Ala Asp Val Gly Asp Asn Leu Asp Ala Asn  
210 215 220

Ile Tyr Gly Asn His Pro Ile Tyr Leu Asp Thr Arg Tyr Phe Thr Lys  
225 230 235 240

Asp Glu Ser Gly Lys Leu Ser Tyr Val Ser Asp Pro Ala Asp Lys Asn  
245 250 255

Ala Lys Tyr Val Ser Tyr Thr Asn Gly Val Phe Leu Arg Asn Ala His  
260 265 270

Ala Gln Glu Val Leu Leu Arg Pro Glu Gly Ile Thr Trp Arg Thr Leu  
275 280 285

Gly Gly Ser Ile Asp Leu Tyr Phe Phe Glu Gly Pro Phe Ala Gln Asp  
290 295 300

Ile Ile Lys Ser Tyr Gln Leu Ser Thr Val Gly Leu Pro Ala Met Gln  
305 310 315 320

Gln Tyr Trp Thr Leu Gly Phe His Gln Cys Arg Trp Gly Tyr Ser Asn  
325 330 335

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Trp Thr Val Val Lys Asp Val Val Asp Asn Phe Arg Lys Phe Gly Ile  
                   340                                  345                                  350

Pro Leu Glu Thr Ile Trp Thr Asp Ile Asp Tyr Met Lys Gly Tyr Arg  
                   355                                  360                                  365

Asp Phe Glu Asn Asp Pro Asp Gln Phe Ser Tyr Glu Glu Gly Ala Arg  
                   370                                  375                                  380

Phe Leu Glu Glu Leu His Lys Asn His Gln His Tyr Val Pro Ile Val  
                   385                                  390                                  395                                  400

Asp Ser Ala Ile Tyr Val Pro Asn Pro Asp Lys Pro Glu Asp Asp Tyr  
                                   405                                  410                                  415

Glu Pro Tyr His Arg Gly Leu Glu Ala Asp Ala Phe Ile Met Asn Pro  
                                   420                                  425                                  430

Asp Gly Ser Leu Tyr Ile Gly Ala Val Trp Pro Gly Tyr Thr Val Phe  
                                   435                                  440                                  445

Pro Asp Trp Ile Gly Ala Ala Leu Asn Gly Thr Gly Thr Val Gly Trp  
                                   450                                  455                                  460

Trp Thr Asp Glu Phe Val Arg Tyr Tyr Lys Lys Val Ala Phe Asp Gly  
                                   465                                  470                                  475                                  480

Ile Trp Ile Asp Met Ser Glu Val Ala Ser Phe Cys Ile Gly Ser Cys  
                                   485                                  490                                  495

Gly Thr Gly Asn Leu Thr Leu Asn Pro Val His Pro Pro Trp Gly Leu  
                                   500                                  505                                  510

Pro Gly Glu Pro Gly Ala Leu Val Leu Asp Tyr Pro Glu Gly Phe Glu  
                                   515                                  520                                  525

Lys Thr Asn Ala Ser Glu Ala Ser Ser Ala Thr Ser Val Tyr Lys Thr  
                                   530                                  535                                  540

Gln Asn Pro Asp Pro Thr Thr Thr Ala Ser Thr Thr Ser Thr Thr Ser  
                                   545                                  550                                  555                                  560

Tyr Leu Arg Thr Thr Pro Thr Pro Gly Val Arg Asn Ile Asn Tyr Pro  
                                   565                                  570                                  575

Pro Tyr Val Ile Asn Asn Phe His Gly Asp Ile Gly Thr His Ala Leu  
                                   580                                  585                                  590

Ser Pro Asn Gly Thr His His Gly Gly Thr Val Asp Tyr Asp Phe His  
                                   595                                  600                                  605

Asn Leu Phe Gly His Gln Ile Leu His Ala Thr Tyr Gln Ala Leu Leu  
                                   610                                  615                                  620

Lys Val Phe Glu Gly Lys Arg Pro Phe Ile Ile Gly Arg Ser Thr Phe  
                                   625                                  630                                  635                                  640

Ala Gly Ser Gly Lys Trp Ala Gly His Trp Gly Gly Asp Asn Tyr Ser  
                                   645                                  650                                  655

Leu Trp Ala Phe Leu Tyr Phe Ser Ile Pro Gln Ala Leu Ser Phe Ser  
                                   660                                  665                                  670

Ile Phe Gly Phe Pro Met Phe Gly Val Asp Thr Cys Gly Phe Asn Gly  
                                   675                                  680                                  685

Asn Thr Asp His Glu Leu Cys Ser Arg Trp Met Gln Leu Ser Ala Phe  
                                   690                                  695                                  700

Phe Pro Phe Tyr Arg Asn His Asn Val Arg Gly Ala Ile Ser Gln Glu  
                                   705                                  710                                  715                                  720

Pro Tyr Val Trp Ser Ser Val Ile Asp Ala Ser Lys Lys Ala Met Arg  
                                   725                                  730                                  735

Ile Arg Tyr Leu Leu Leu Pro Tyr Met Tyr Thr Leu Met Ala Gln Ala  
                                   740                                  745                                  750

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Ser Leu Ser Gly Asp Thr Val Met Arg Ala Leu Ser Trp Glu Phe Pro  
 755 760 765  
 Gln Glu Pro Trp Leu Ala Asp Ala Asp Arg Gln Phe Met Leu Gly Ser  
 770 775 780  
 Ala Val Met Val Thr Pro Cys Leu Val Gln Gly Ala Asn Thr Val Asp  
 785 790 795 800  
 Gly Val Phe Pro Gly Val Gly Asp Gly Thr Ile Trp Tyr Asp Trp Tyr  
 805 810 815  
 Thr Tyr Lys Ala Ala Ser Glu Gly Val Gln Pro Gly Glu Asn Val Thr  
 820 825 830  
 Ile Asp Ala Pro Leu Gly His Ile Pro Val Phe Leu Arg Gly Gly His  
 835 840 845  
 Val Ile Pro Val Gln Glu Pro Gly Met Thr Thr Thr Glu Ser Arg Gln  
 850 855 860  
 Asn Glu Trp Ser Val Ile Val Ala Leu Asp Gly Ala Gly Lys Ala Asn  
 865 870 875 880  
 Gly Thr Leu Tyr Leu Asp Asp Gly Glu Ser Leu Glu Pro Gly Glu Asn  
 885 890 895  
 Val Lys Trp Val Asp Phe Thr Val Glu Lys Asn Ser Phe Arg Val Thr  
 900 905 910  
 Pro Gln Gly Lys Tyr Leu Asp Arg Asn Ser Leu Ala Asn Val Thr Ile  
 915 920 925  
 Leu Gly Val Ala Glu Ala Pro Leu Gly Val Ala Ile Asn Ser His Leu  
 930 935 940  
 Leu Gly Ser Ala Ser Trp Ser Tyr Asp Ser Glu Gly Lys Phe Leu Ser  
 945 950 955 960  
 Val Thr Glu Leu Gln Asp Asn Phe Lys Glu Gly Ala Trp Ala Ser Asn  
 965 970 975  
 Trp Thr Leu Ser Trp Asn Ser Ala Ser Asn Ser Gly Ser Ser Pro Val  
 980 985 990  
 Gln Gly Gly Gly Gly Arg Leu Glu Phe Ser Thr Pro Asn Leu Leu His  
 995 1000 1005  
 Ala Ala Ala Phe Gly Ile Leu Phe Gly Arg Met Phe Val Val  
 1010 1015 1020

&lt;210&gt; SEQ ID NO 13

&lt;211&gt; LENGTH: 3293

&lt;212&gt; TYPE: DNA

<213> ORGANISM: *Rasamsonia composticola*

&lt;400&gt; SEQUENCE: 13

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atgcggccaa cttccctcgt caagcacttg gctgcgacca gcctcctctt tctcgcggcg      60
gatgcagctg ctatcgtccg ccgcaacggc gcctctcctt catgccccgg ctataaagcg      120
agtaacgtga agaccgtcga cggtgaaatc gtcagcgcgg atctcaatct cgcgggtccc      180
gcctgcaatg tgtatggcac ggatctggac gatctgaagc tgcaggttga gtaccaatca      240
ggtaagtgcg acagcatcgc cgtctgcagc ttatcggcag catgtgatcg ggcctggac      300
tagggcttag ggctgggggt tagggctgac tgcttgctgc cgttcgatgt gactggaact      360
gtacggttgg ttgacaacga tctgacatct gcagaacaac gcctccatgt gaagatctac      420
gatgccgccc agcaggtcta ccaggtgccc accgcggtgc tccccggcc cagcagcggc      480
aacatcccc cggccaagtc ggacctgaag ttctccatga ccaacgaccc cttctccttt      540
accatcaagc gcagatcaaa cggcgaaatc ctcttcgaca cctccggcca tccgctgatc      600

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ttcgagtcgc agtatctggg cctccgtacc aagctgccgg actcgcccaa catctacggc	660
ctgggagagc acaccgggtc tttccgctcg cccaccaaga attacaccgg cacgctgtgg	720
tcgcgcatg cgtacggtac gcccaagac accaacctgt acggcaacca cccggtgtac	780
ttcgactacc gcggcagcaa cggcaccat ggcgtgtcc tgctgaacag caacggcatg	840
gacgtcgata tcgacgtcga ctccgacgga cagtacctgc agtacaacac cctggggggc	900
gtgctggact tctacttctc cagcgggccc gateccaagg ccgtcgcgac gcagtatgcc	960
gagacggtcg gaaaaccggc catgatgcc tactggggat tcggcttcca caactgcaga	1020
tatggatacc aggacatcta tgaggttgct gagatcattg ccaactacag tgccgcaaac	1080
attccgcttg agaccaatg gactgatatc ggtatgcttt ccatcccggg gccgtggttt	1140
ttgcttctca gcgtggctga ctgttcgaga ctatatgat ctgaggaaag tgtttacgct	1200
ggaccctat cgctatccat tgaagctcgt ccaagaggtt gtctcttacc tccacaagca	1260
caaccagcac tacatcatga tgggggacct tgcagtggca taccagaact attcagcgtt	1320
caacaacggc gtcgctgccg acgctttctc gaagtctcga aatggctcca tctaccaggg	1380
tgctgtctgg ccggggccga cggcgttccc ggactgggtc gcacccaga cacaggagt	1440
ttggaatagc gagttctcga ccttctttga ccccgccac gggtcgcaca tcgatgccct	1500
ttggatcgac atgaacgagg cgtccaactt ctgcgacttt ccctgctcga accccgccgc	1560
gtatgcggca gccaacggcg atccgccac gccctccgccc gtccgcttga gcccccgag	1620
gccgattcct ggatttggcc ctgacttcca gccgacgtgt gtcgccacgg tgcgttcca	1680
ttgcgatcgc cagacctact ttggcgagaa catcctcacc ctgggtaact cgacgacact	1740
gggagccggc gacgttcaca tggcgcagc catgagcgc aacaactacc cgatctggca	1800
gctgaccgtc cagatgcgc cgaatgggac gttctcgtac cagtacgttc gcaaggaatc	1860
ggacggcagt tacatctacg aacagacgaa tcgcacggtc acgacgggag actgcaccag	1920
cgacacgctt aaggtgtccg acaccatcac caccagctct ggaccgcaca agagatccga	1980
attacggcgc ctggtgcgct cgcggttccc ggccggaggac ctgaccaggc gccagtctgg	2040
atcgatgttg ggcctgccc acaggaacct gctgaatccg ccatacacca tccacaatgc	2100
ggctggcaac ctgagtgaga agaccatcaa caccgacctg atccatgcgg gcggatatgc	2160
cgagtacgac acgcacaact tgtacggcac gatgatgagc gcgaccagca gggaggcgat	2220
gctgaaccgc agaccagcag tcaggccact tgtgtaagtc atccatcgtc cttaagccag	2280
acacagcatg ttaggggcta acgggcagta gcattaccgg gtcgacctc gctggagccg	2340
gccgacaggt cggccactgg ctccggcaca atttcgccga ttggggaccac taccggtgga	2400
cgatcgcga gctgcaggaa ttcgcccgc tgttccagat cccgatggc gccagcgaca	2460
tctcgggta cgaaggcaac acgacggaca acctgtgctc gcgctgggtc ttctcggcg	2520
ccttctcgc cttcttcgc gaccactcgg acaaccagtc gccgccgac gagctgtacc	2580
gcactccgca gatcgcggcg gccgcgccc cgcaccatga catccgctac cgtctgctcg	2640
actacgcta cacggtgctg tggacgcaga cccagaccgg cgcgccgatg ctcaacccca	2700
tgttctcga gtaccggcc gacagcaaca ccgccacct gcagtaaccag ttcttctggg	2760
gcgacagcat catggtcgcg cccgtgaccg acaacgactc gaccaccgtc aacgtctact	2820
tcccgaagga ccagttctac gacttctaca ccggcgacc tgtgtccggg gagggcaata	2880
ccgtcacct gaccgacgtc ggtctcga caatcccgt gtacttcaag ggcgggagca	2940
tcgtgccat gcgctgcgc tcggcgaaca cgacggcgga gctgcggcag caggacttcg	3000

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tcgtcgatcgat cgccccggac agccacggcg acgcgacggg ccagctgtac ctgacgacg 3060
gcgagagcat caaccagccg cacaccagcg agatccagtt ctctaccgc ggaggccatt 3120
tcagcatgac aggcaagttt gactatgatc ccggcaacgt ggatcatcagc cagatcacgc 3180
tgctgggtgc ggacggcgcc ggtaaagggg gtctgtataa cagcaccacc aaggtggcga 3240
cctacaaagt caacgcgaag ttgacgggta aattcgaagc cagcttacac taa 3293

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&lt;210&gt; SEQ ID NO 14

&lt;211&gt; LENGTH: 1035

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Rasamsonia composticola*

&lt;400&gt; SEQUENCE: 14

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Met Arg Pro Thr Ser Leu Val Lys His Leu Ala Ala Thr Ser Leu Leu
1          5          10          15
Phe Leu Ala Ala Asp Ala Ala Ala Ile Val Arg Arg Asn Gly Ala Ser
20          25          30
Pro Ser Cys Pro Gly Tyr Lys Ala Ser Asn Val Lys Thr Val Asp Gly
35          40          45
Glu Ile Val Ser Ala Asp Leu Asn Leu Ala Gly Pro Ala Cys Asn Val
50          55          60
Tyr Gly Thr Asp Leu Asp Asp Leu Lys Leu Gln Val Glu Tyr Gln Ser
65          70          75          80
Gly Pro Gly Val Arg Ala Asp Cys Leu Leu Pro Phe Asp Val Thr Gly
85          90          95
Thr Val Arg Leu Val Asp Asn Asp Leu Thr Ser Ala Glu Gln Arg Leu
100         105         110
His Val Lys Ile Tyr Asp Ala Ala Glu Gln Val Tyr Gln Val Pro Thr
115         120         125
Ala Val Leu Pro Arg Pro Ser Ser Ala Asn Ile Pro Pro Ala Lys Ser
130         135         140
Asp Leu Lys Phe Ser Met Thr Asn Asp Pro Phe Ser Phe Thr Ile Lys
145         150         155         160
Arg Arg Ser Asn Gly Glu Ile Leu Phe Asp Thr Ser Gly His Pro Leu
165         170         175
Ile Phe Glu Ser Gln Tyr Leu Gly Leu Arg Thr Lys Leu Pro Asp Ser
180         185         190
Pro Asn Ile Tyr Gly Leu Gly Glu His Thr Gly Ser Phe Arg Leu Pro
195         200         205
Thr Lys Asn Tyr Thr Arg Thr Leu Trp Ser Arg Asp Ala Tyr Gly Thr
210         215         220
Pro Lys Asp Thr Asn Leu Tyr Gly Asn His Pro Val Tyr Phe Asp Tyr
225         230         235         240
Arg Gly Ser Asn Gly Thr His Gly Val Phe Leu Leu Asn Ser Asn Gly
245         250         255
Met Asp Val Asp Ile Asp Val Asp Ser Asp Gly Gln Tyr Leu Gln Tyr
260         265         270
Asn Thr Leu Gly Gly Val Leu Asp Phe Tyr Phe Leu Ser Gly Pro Asp
275         280         285
Pro Lys Ala Val Ala Thr Gln Tyr Ala Glu Thr Val Gly Lys Pro Val
290         295         300
Met Met Pro Tyr Trp Gly Phe Gly Phe His Asn Cys Arg Tyr Gly Tyr
305         310         315         320

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Gln Asp Ile Tyr Glu Val Ala Glu Ile Ile Ala Asn Tyr Ser Ala Ala  
 325 330 335  
 Asn Ile Pro Leu Glu Thr Gln Trp Thr Asp Ile Asp Tyr Met Asp Leu  
 340 345 350  
 Arg Lys Val Phe Thr Leu Asp Pro Tyr Arg Tyr Pro Leu Lys Leu Val  
 355 360 365  
 Gln Glu Val Val Ser Tyr Leu His Lys His Asn Gln His Tyr Ile Met  
 370 375 380  
 Met Val Asp Pro Ala Val Ala Tyr Gln Asn Tyr Ser Ala Phe Asn Asn  
 385 390 395 400  
 Gly Val Ala Ala Asp Ala Phe Leu Lys Phe Ser Asn Gly Ser Ile Tyr  
 405 410 415  
 Gln Gly Val Val Trp Pro Gly Pro Thr Ala Phe Pro Asp Trp Phe Ala  
 420 425 430  
 Pro Gln Thr Gln Glu Phe Trp Asn Ser Glu Phe Ser Thr Phe Phe Asp  
 435 440 445  
 Pro Ala His Gly Val Asp Ile Asp Ala Leu Trp Ile Asp Met Asn Glu  
 450 455 460  
 Ala Ser Asn Phe Cys Asp Phe Pro Cys Ser Asn Pro Ala Ala Tyr Ala  
 465 470 475 480  
 Ala Ala Asn Gly Asp Pro Pro Thr Pro Pro Val Arg Leu Ser Pro  
 485 490 495  
 Pro Arg Pro Ile Pro Gly Phe Gly Pro Asp Phe Gln Pro Thr Cys Val  
 500 505 510  
 Ala Thr Val Ser Phe Asp Cys Asp Ala Gln Thr Tyr Phe Gly Glu Asn  
 515 520 525  
 Ile Leu Ile Leu Gly Asn Ser Thr Thr Leu Gly Ala Gly Asp Val His  
 530 535 540  
 Met Ala Pro Val Met Ser Ala Asn Asn Tyr Pro Ile Trp Gln Leu Thr  
 545 550 555 560  
 Val Gln Met Pro Pro Asn Gly Thr Phe Ser Tyr Gln Tyr Val Arg Lys  
 565 570 575  
 Glu Ser Asp Gly Ser Tyr Ile Tyr Glu Gln Thr Asn Arg Thr Val Thr  
 580 585 590  
 Thr Gly Asp Cys Thr Ser Gly Thr Leu Lys Val Ser Asp Thr Ile Thr  
 595 600 605  
 Thr Ser Ser Gly Pro His Lys Arg Ser Glu Leu Arg Pro Leu Val Arg  
 610 615 620  
 Ser Pro Phe Pro Ala Glu Asp Leu Thr Arg Arg Gln Ser Gly Ser Met  
 625 630 635 640  
 Leu Gly Leu Pro Asn Arg Asn Leu Leu Asn Pro Pro Tyr Thr Ile His  
 645 650 655  
 Asn Ala Ala Gly Asn Leu Ser Glu Lys Thr Ile Asn Thr Asp Leu Ile  
 660 665 670  
 His Ala Gly Gly Tyr Ala Glu Tyr Asp Thr His Asn Leu Tyr Gly Thr  
 675 680 685  
 Met Met Ser Ala Thr Ser Arg Glu Ala Met Leu Asn Arg Arg Pro Ala  
 690 695 700  
 Val Arg Pro Leu Val Ile Thr Arg Ser Thr Phe Ala Gly Ala Gly Arg  
 705 710 715 720  
 Gln Val Gly His Trp Leu Gly Asp Asn Phe Ala Asp Trp Asp His Tyr  
 725 730 735  
 Arg Trp Thr Ile Ala Glu Leu Gln Glu Phe Ala Ala Leu Phe Gln Ile

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740					745					750					
Pro	Met	Val	Gly	Ser	Asp	Ile	Cys	Gly	Tyr	Asp	Gly	Asn	Thr	Thr	Asp
		755					760					765			
Asn	Leu	Cys	Ser	Arg	Trp	Val	Phe	Leu	Gly	Ala	Phe	Ser	Pro	Phe	Phe
	770					775					780				
Arg	Asp	His	Ser	Asp	Asn	Gln	Ser	Pro	Pro	His	Glu	Leu	Tyr	Arg	Thr
	785				790					795					800
Pro	Gln	Ile	Ala	Ala	Ala	Ala	Arg	Ala	Ala	Ile	Asp	Ile	Arg	Tyr	Arg
				805					810					815	
Leu	Leu	Asp	Tyr	Ala	Tyr	Thr	Val	Leu	Trp	Thr	Gln	Thr	Gln	Thr	Gly
		820						825					830		
Ala	Pro	Met	Leu	Asn	Pro	Met	Phe	Phe	Glu	Tyr	Pro	Ala	Asp	Ser	Asn
		835					840					845			
Thr	Ala	Asp	Leu	Gln	Tyr	Gln	Phe	Phe	Trp	Gly	Asp	Ser	Ile	Met	Val
	850					855					860				
Ala	Pro	Val	Thr	Asp	Asn	Asp	Ser	Thr	Thr	Val	Asn	Val	Tyr	Phe	Pro
	865				870					875					880
Lys	Asp	Gln	Phe	Tyr	Asp	Phe	Tyr	Thr	Gly	Ala	Pro	Val	Ser	Gly	Glu
			885						890					895	
Gly	Asn	Thr	Val	Thr	Leu	Thr	Asp	Val	Gly	Phe	Asp	Thr	Ile	Pro	Leu
		900						905					910		
Tyr	Phe	Lys	Gly	Gly	Ser	Ile	Val	Pro	Met	Arg	Val	Arg	Ser	Ala	Asn
		915					920					925			
Thr	Thr	Ala	Glu	Leu	Arg	Gln	Gln	Asp	Phe	Val	Val	Val	Ile	Ala	Pro
	930					935					940				
Asp	Ser	His	Gly	Asp	Ala	Thr	Gly	Gln	Leu	Tyr	Leu	Asp	Asp	Gly	Glu
	945				950					955					960
Ser	Ile	Asn	Gln	Pro	His	Thr	Ser	Glu	Ile	Gln	Phe	Ser	Tyr	Arg	Gly
			965						970					975	
Gly	His	Phe	Ser	Met	Thr	Gly	Lys	Phe	Asp	Tyr	Asp	Pro	Gly	Asn	Val
		980						985					990		
Val	Ile	Ser	Gln	Ile	Thr	Leu	Leu	Gly	Ala	Asp	Gly	Ala	Gly	Lys	Gly
		995					1000					1005			
Gly	Ser	Tyr	Asn	Ser	Thr	Thr	Lys	Val	Ala	Thr	Tyr	Lys	Val	Asn	
	1010					1015					1020				
Ala	Lys	Leu	Thr	Gly	Lys	Phe	Glu	Ala	Ser	Leu	His				
	1025					1030					1035				

&lt;210&gt; SEQ ID NO 15

&lt;211&gt; LENGTH: 1013

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Rasamsonia composticola

&lt;400&gt; SEQUENCE: 15

Ala	Ala	Ile	Val	Arg	Arg	Asn	Gly	Ala	Ser	Pro	Ser	Cys	Pro	Gly	Tyr
1				5					10					15	
Lys	Ala	Ser	Asn	Val	Lys	Thr	Val	Asp	Gly	Glu	Ile	Val	Ser	Ala	Asp
		20						25					30		
Leu	Asn	Leu	Ala	Gly	Pro	Ala	Cys	Asn	Val	Tyr	Gly	Thr	Asp	Leu	Asp
		35					40					45			
Asp	Leu	Lys	Leu	Gln	Val	Glu	Tyr	Gln	Ser	Gly	Pro	Gly	Val	Arg	Ala
	50					55					60				
Asp	Cys	Leu	Leu	Pro	Phe	Asp	Val	Thr	Gly	Thr	Val	Arg	Leu	Val	Asp
	65				70					75					80



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500					505					510					
Ser	Thr	Thr	Leu	Gly	Ala	Gly	Asp	Val	His	Met	Ala	Pro	Val	Met	Ser
	515						520					525			
Ala	Asn	Asn	Tyr	Pro	Ile	Trp	Gln	Leu	Thr	Val	Gln	Met	Pro	Pro	Asn
	530					535					540				
Gly	Thr	Phe	Ser	Tyr	Gln	Tyr	Val	Arg	Lys	Glu	Ser	Asp	Gly	Ser	Tyr
	545				550					555					560
Ile	Tyr	Glu	Gln	Thr	Asn	Arg	Thr	Val	Thr	Thr	Gly	Asp	Cys	Thr	Ser
				565					570						575
Gly	Thr	Leu	Lys	Val	Ser	Asp	Thr	Ile	Thr	Thr	Ser	Ser	Gly	Pro	His
			580						585					590	
Lys	Arg	Ser	Glu	Leu	Arg	Pro	Leu	Val	Arg	Ser	Pro	Phe	Pro	Ala	Glu
		595					600					605			
Asp	Leu	Thr	Arg	Arg	Gln	Ser	Gly	Ser	Met	Leu	Gly	Leu	Pro	Asn	Arg
	610					615					620				
Asn	Leu	Leu	Asn	Pro	Pro	Tyr	Thr	Ile	His	Asn	Ala	Ala	Gly	Asn	Leu
	625				630					635					640
Ser	Glu	Lys	Thr	Ile	Asn	Thr	Asp	Leu	Ile	His	Ala	Gly	Gly	Tyr	Ala
				645						650					655
Glu	Tyr	Asp	Thr	His	Asn	Leu	Tyr	Gly	Thr	Met	Met	Ser	Ala	Thr	Ser
			660						665					670	
Arg	Glu	Ala	Met	Leu	Asn	Arg	Arg	Pro	Ala	Val	Arg	Pro	Leu	Val	Ile
		675					680					685			
Thr	Arg	Ser	Thr	Phe	Ala	Gly	Ala	Gly	Arg	Gln	Val	Gly	His	Trp	Leu
	690					695					700				
Gly	Asp	Asn	Phe	Ala	Asp	Trp	Asp	His	Tyr	Arg	Trp	Thr	Ile	Ala	Glu
	705				710					715					720
Leu	Gln	Glu	Phe	Ala	Ala	Leu	Phe	Gln	Ile	Pro	Met	Val	Gly	Ser	Asp
				725						730					735
Ile	Cys	Gly	Tyr	Asp	Gly	Asn	Thr	Thr	Asp	Asn	Leu	Cys	Ser	Arg	Trp
			740						745					750	
Val	Phe	Leu	Gly	Ala	Phe	Ser	Pro	Phe	Phe	Arg	Asp	His	Ser	Asp	Asn
		755					760					765			
Gln	Ser	Pro	Pro	His	Glu	Leu	Tyr	Arg	Thr	Pro	Gln	Ile	Ala	Ala	Ala
	770					775					780				
Ala	Arg	Ala	Ala	Ile	Asp	Ile	Arg	Tyr	Arg	Leu	Leu	Asp	Tyr	Ala	Tyr
	785				790					795					800
Thr	Val	Leu	Trp	Thr	Gln	Thr	Gln	Thr	Gly	Ala	Pro	Met	Leu	Asn	Pro
				805						810					815
Met	Phe	Phe	Glu	Tyr	Pro	Ala	Asp	Ser	Asn	Thr	Ala	Asp	Leu	Gln	Tyr
			820					825					830		
Gln	Phe	Phe	Trp	Gly	Asp	Ser	Ile	Met	Val	Ala	Pro	Val	Thr	Asp	Asn
		835					840						845		
Asp	Ser	Thr	Thr	Val	Asn	Val	Tyr	Phe	Pro	Lys	Asp	Gln	Phe	Tyr	Asp
	850					855						860			
Phe	Tyr	Thr	Gly	Ala	Pro	Val	Ser	Gly	Glu	Gly	Asn	Thr	Val	Thr	Leu
	865				870					875					880
Thr	Asp	Val	Gly	Phe	Asp	Thr	Ile	Pro	Leu	Tyr	Phe	Lys	Gly	Gly	Ser
				885					890						895
Ile	Val	Pro	Met	Arg	Val	Arg	Ser	Ala	Asn	Thr	Thr	Ala	Glu	Leu	Arg
			900						905						910
Gln	Gln	Asp	Phe	Val	Val	Val	Ile	Ala	Pro	Asp	Ser	His	Gly	Asp	Ala
			915						920					925	

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Thr Gly Gln Leu Tyr Leu Asp Asp Gly Glu Ser Ile Asn Gln Pro His  
 930 935 940  
 Thr Ser Glu Ile Gln Phe Ser Tyr Arg Gly Gly His Phe Ser Met Thr  
 945 950 955 960  
 Gly Lys Phe Asp Tyr Asp Pro Gly Asn Val Val Ile Ser Gln Ile Thr  
 965 970 975  
 Leu Leu Gly Ala Asp Gly Ala Gly Lys Gly Gly Ser Tyr Asn Ser Thr  
 980 985 990  
 Thr Lys Val Ala Thr Tyr Lys Val Asn Ala Lys Leu Thr Gly Lys Phe  
 995 1000 1005  
 Glu Ala Ser Leu His  
 1010

&lt;210&gt; SEQ ID NO 16

&lt;211&gt; LENGTH: 3162

&lt;212&gt; TYPE: DNA

<213> ORGANISM: *Rasamsonia composticola*

&lt;400&gt; SEQUENCE: 16

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atggcaggct cgcgcccoct tgttgccagc ctcgtctggc ttgctcaggc cttcgacgct    60
cttgcaggac cggtcagcag tacgactgcc gcagcaccat ctgctcaatt cacgctcccg    120
gccgctgctg atgttggggc caacttgctt gccaacatcg acgatcccaa tgccgtcaac    180
gcccaggatg tctgtcccgg ttacacggcg tcgaacgtgc agaacaccga gtctgggttt    240
gtggcgaccc tgacgctggc ggggaaacca tgtaatgtgt acggaacgga cgtggagtcc    300
ctgaacctga cggttgagta ccaagctgct gatcgactga acatcaatat cgtcccagc    360
cacgctgatt cttcaaacca gtcgtgggat ctgcttcccg aaaatgtagt gcccaaaccc    420
ggggtcgatg caggagccca agtcccggag agtgatctcg tcttcagctg gtcgaatgaa    480
ccctccttca atttcaaggt gatccggaaa gccacaggcg acattctctt cgacacggag    540
ggttctgtcc tgggtgttoga aaaccagttc atcgagtttg cgagcgtctt gccggagaac    600
tacaatctct acggtctggg agagcgtatc catggcctgc gactggggaa caacttcacc    660
gccacgacgt atgccgcgga tagcgcagac cctattgacc ggtgagtatc tgagatcgac    720
tgctcagctc gctctgttgg atctgaaaga agttataaaa ctgacctagc tcaggaacat    780
ctacgggacc catccctttt atctggacac ccggtactac gaggttgatt ccgagcatgg    840
gaggttcaog ttggtgacgg acaacgagac cgatttctcc aaggaatata tgtcgctctc    900
gcatggagtt ttcctgagaa atgccacgg acaggaggtg ctgctgcgct ctcagagcat    960
cacctggcgg aactcgggtg gcagcattga tctttacttc tacgcccggc cgaccaggc    1020
cgatgttacc cgcagctacc agaccagcac cgttggcctc ccggcaatgc agcagtactt    1080
caccctgggc tatcatcagt gccgctgggg atacagaaac tggtcggagc tagctgatgt    1140
agtggccaat ttcagaaat tcgagatccc attgaaaaat atctggtaag gcatacgtca    1200
tctgaaagag ttgctgggaa agtgatctga caacttcgct tctccaggtc ggatattgat    1260
tacatgaaog agtaccgoga ctttgagaac gaccgggttc gcttctccta cagcgaggga    1320
gcccatttcc tggaccagct ccacaagagt ggccgtcact acatcccgat tgtggacgcc    1380
gcgatctatg accccaacc taacaatgac tccgacgcgt aagtctagtc ttgtaggag    1440
gtgataggga gtggagctga cttctcgatt aggtatgcga catatgatcg aggttctaag    1500
gacgatctct ggttgaagaa tcccagcggc agcgtgtaca tcggagccgt ctggcctggc    1560

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tacacagtgt tcaccgattg gcaccatcca aaagccaacg agtgggtgggc aaacgagctg 1620
gctctgtggc acgaaaaggt cgcttttgac ggaatctggc tggacatgaa cgaggctctcg 1680
tccttctcgg ttggcagctg tggaacaggg aacctgacct tgaatcccg tgcacccgaa 1740
ttcgcgctcc cgggagagcc tggagctgtc atctacgact accccgagga cttcaacgtg 1800
acgaatgcc a cggcgggcgc gtctgcatct gccgcgtcct cgagccaagc tgctgcgaca 1860
gagacageta cttctctgct cagcactacc agctacctgg tgaccacgcc cactcctgga 1920
gtgcggaatg tcaactaacc tccctatgtg attaatacag tgcaggaggg tcaagatctc 1980
gctgttcacg ccgtctcgcc caacgcaacc catgtcogat gtgtgcagga gtacgacgtg 2040
cacaatctct ggggctacca ggagacaaat gcaacctacc atgccctgct gagcatcttc 2100
cccgggaaga gaccgttcat catctcccg tccacgttcg ccggcagcgg cagatgggcc 2160
ggacactggg gtggcgacaa cgctcgaaa tgggcgtaca tgttcttttc tatcccgcag 2220
gagctatcgt tctcgtggtt cggcatcccc atgttcggcg tcgacacctg cgggttcaac 2280
ggcaactcgg acgaagagct gtgcaaccgc tggatgcagc tctccgcctt cttccccttc 2340
taccgcaacc acaacgtcct gtccggccatc ccgcaggagc cctatgtctg ggcatccgtc 2400
atcgaggcga gcaagtggc aatgaggatc cgctacacct tgctccetta cctctacaca 2460
ctgttctacc tcgcccacac cacggggtcg accgtcatgc gtgccttggc gtgggagttc 2520
cccaacgacc cgtccctcgc tgcctgggac cggcagttcc tccctggccc gtcgctgatg 2580
gtcgtccccg tgctcgagcc gcaggctgat accgtcaagg gcgtcttccc gggcgttgcc 2640
cagggccaag tctggtacga ctggtacacg cagaccgcgt tcgacgcgca gccaggcgtg 2700
aacacgacca tctccgcgcc gctgggccac atccccgtgt tcgtccgcgg cgggagcgtg 2760
ctccccatgc agcagccggc actggtgacg cgggacgtgc gcaacagccc ctggtcgtg 2820
ctggtcgcgc tgggcagcga cggcacggcc tcgggacagc tgtacgtgga cgacggcgag 2880
agcatcacac ctccggcgtc cctgcacgtc gacttcgtgg cggccaactt ctcgaccctc 2940
ttcgcgacgg ccccggtgtc gttcaaggac agcaaacgcg tggctaacgt cacgggtgctg 3000
ggcgtcccag ccgcgccgtc gtctgcagtc acttggaaac acgagacggt tccttcggag 3060
tcggtgctgt acaatgccac ctccaaagtc ctctgggtca atggactgca gagtcttacc 3120
cgtgacggag cctgggagcag tgactggggt ctgaagtgggt aa 3162

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&lt;210&gt; SEQ ID NO 17

&lt;211&gt; LENGTH: 990

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Rasamsonia composticola*

&lt;400&gt; SEQUENCE: 17

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Met Ala Gly Ser Ala Ala Leu Val Ala Ser Leu Val Trp Leu Ala Gln
 1           5           10          15

Ala Phe Asp Ala Leu Ala Gly Pro Val Ser Ser Thr Thr Ala Ala Ala
 20          25          30

Pro Ser Ala Gln Phe Thr Val Pro Ala Ala Ala Asp Val Gly Ala Asn
 35          40          45

Leu Leu Ala Asn Ile Asp Asp Pro Asn Ala Val Asn Ala Gln Asp Val
 50          55          60

Cys Pro Gly Tyr Thr Ala Ser Asn Val Gln Asn Thr Glu Ser Gly Phe
 65          70          75          80

Val Ala Thr Leu Thr Leu Ala Gly Lys Pro Cys Asn Val Tyr Gly Thr
 85          90          95

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Asp Val Glu Ser Leu Asn Leu Thr Val Glu Tyr Gln Ala Ala Asp Arg  
                   100  105  110

Leu Asn Ile Asn Ile Val Pro Thr His Val Asp Ser Ser Asn Gln Ser  
                   115  120  125

Trp Tyr Leu Leu Pro Glu Asn Val Val Pro Lys Pro Gly Val Asp Ala  
                   130  135  140

Gly Ala Gln Val Pro Glu Ser Asp Leu Val Phe Ser Trp Ser Asn Glu  
                   145  150  155  160

Pro Ser Phe Asn Phe Lys Val Ile Arg Lys Ala Thr Gly Asp Ile Leu  
                                   165  170  175

Phe Asp Thr Glu Gly Ser Val Leu Val Phe Glu Asn Gln Phe Ile Glu  
                                   180  185  190

Phe Ala Ser Ala Leu Pro Glu Asn Tyr Asn Leu Tyr Gly Leu Gly Glu  
                                   195  200  205

Arg Ile His Gly Leu Arg Leu Gly Asn Asn Phe Thr Ala Thr Thr Tyr  
                   210  215  220

Ala Ala Asp Ser Ala Asp Pro Ile Asp Arg Asn Ile Tyr Gly Thr His  
                   225  230  235  240

Pro Phe Tyr Leu Asp Thr Arg Tyr Tyr Glu Val Asp Ser Glu His Gly  
                                   245  250  255

Arg Phe Thr Leu Val Thr Asp Asn Glu Thr Asp Phe Ser Lys Glu Tyr  
                                   260  265  270

Leu Ser Leu Ser His Gly Val Phe Leu Arg Asn Ala His Gly Gln Glu  
                   275  280  285

Val Leu Leu Arg Pro Gln Ser Ile Thr Trp Arg Thr Leu Gly Gly Ser  
                   290  295  300

Ile Asp Leu Tyr Phe Tyr Ala Gly Pro Thr Gln Ala Asp Val Thr Arg  
                   305  310  315  320

Ser Tyr Gln Thr Ser Thr Val Gly Leu Pro Ala Met Gln Gln Tyr Phe  
                                   325  330  335

Thr Leu Gly Tyr His Gln Cys Arg Trp Gly Tyr Arg Asn Trp Ser Glu  
                                   340  345  350

Leu Ala Asp Val Val Ala Asn Phe Glu Lys Phe Glu Ile Pro Leu Glu  
                   355  360  365

Asn Ile Trp Ser Asp Ile Asp Tyr Met Asn Glu Tyr Arg Asp Phe Glu  
                   370  375  380

Asn Asp Pro Val Arg Phe Ser Tyr Ser Glu Gly Ala Lys Phe Leu Asp  
                   385  390  395  400

Gln Leu His Lys Ser Gly Arg His Tyr Ile Pro Ile Val Asp Ala Ala  
                                   405  410  415

Ile Tyr Asp Pro Asn Pro Asn Asn Asp Ser Asp Ala Tyr Ala Thr Tyr  
                                   420  425  430

Asp Arg Gly Ser Lys Asp Asp Ile Trp Leu Lys Asn Pro Asp Gly Ser  
                   435  440  445

Val Tyr Ile Gly Ala Val Trp Pro Gly Tyr Thr Val Phe Thr Asp Trp  
                   450  455  460

His His Pro Lys Ala Asn Glu Trp Trp Ala Asn Glu Leu Ala Leu Trp  
                   465  470  475  480

His Glu Lys Val Ala Phe Asp Gly Ile Trp Leu Asp Met Asn Glu Val  
                                   485  490  495

Ser Ser Phe Cys Val Gly Ser Cys Gly Thr Gly Asn Leu Thr Leu Asn  
                   500  505  510

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Pro Val His Pro Asn Phe Ala Leu Pro Gly Glu Pro Gly Ala Val Ile  
 515 520 525  
 Tyr Asp Tyr Pro Glu Asp Phe Asn Val Thr Asn Ala Thr Ala Ala Ala  
 530 535 540  
 Ser Ala Ser Ala Ala Ser Ser Ser Gln Ala Ala Ala Thr Ala Thr Ala  
 545 550 555 560  
 Thr Ser Ser Ser Thr Thr Thr Ser Tyr Leu Val Thr Thr Pro Thr Pro  
 565 570 575  
 Gly Val Arg Asn Val Asn Tyr Pro Pro Tyr Val Ile Asn His Val Gln  
 580 585 590  
 Glu Gly His Asp Leu Ala Val His Ala Val Ser Pro Asn Ala Thr His  
 595 600 605  
 Val Asp Gly Val Gln Glu Tyr Asp Val His Asn Leu Trp Gly Tyr Gln  
 610 615 620  
 Glu Thr Asn Ala Thr Tyr His Ala Leu Leu Ser Ile Phe Pro Gly Lys  
 625 630 635 640  
 Arg Pro Phe Ile Ile Ser Arg Ser Thr Phe Ala Gly Ser Gly Arg Trp  
 645 650 655  
 Ala Gly His Trp Gly Gly Asp Asn Ala Ser Lys Trp Ala Tyr Met Phe  
 660 665 670  
 Phe Ser Ile Pro Gln Ala Leu Ser Phe Ser Leu Phe Gly Ile Pro Met  
 675 680 685  
 Phe Gly Val Asp Thr Cys Gly Phe Asn Gly Asn Ser Asp Glu Glu Leu  
 690 695 700  
 Cys Asn Arg Trp Met Gln Leu Ser Ala Phe Phe Pro Phe Tyr Arg Asn  
 705 710 715 720  
 His Asn Val Leu Ser Ala Ile Pro Gln Glu Pro Tyr Val Trp Ala Ser  
 725 730 735  
 Val Ile Glu Ala Ser Lys Ser Ala Met Arg Ile Arg Tyr Thr Leu Leu  
 740 745 750  
 Pro Tyr Leu Tyr Thr Leu Phe Tyr Leu Ala His Thr Thr Gly Ser Thr  
 755 760 765  
 Val Met Arg Ala Leu Ala Trp Glu Phe Pro Asn Asp Pro Ser Leu Ala  
 770 775 780  
 Ala Val Asp Arg Gln Phe Leu Leu Gly Pro Ser Leu Met Val Val Pro  
 785 790 795 800  
 Val Leu Glu Pro Gln Val Asp Thr Val Lys Gly Val Phe Pro Gly Val  
 805 810 815  
 Ala Gln Gly Gln Val Trp Tyr Asp Trp Tyr Thr Gln Thr Ala Phe Asp  
 820 825 830  
 Ala Gln Pro Gly Val Asn Thr Thr Ile Ser Ala Pro Leu Gly His Ile  
 835 840 845  
 Pro Val Phe Val Arg Gly Gly Ser Val Leu Pro Met Gln Gln Pro Ala  
 850 855 860  
 Leu Val Thr Arg Asp Val Arg Asn Ser Pro Trp Ser Leu Leu Val Ala  
 865 870 875 880  
 Leu Gly Ser Asp Gly Thr Ala Ser Gly Gln Leu Tyr Val Asp Asp Gly  
 885 890 895  
 Glu Ser Ile Thr Pro Pro Ala Ser Leu His Val Asp Phe Val Ala Ala  
 900 905 910  
 Asn Phe Ser Thr Leu Phe Ala Thr Ala Arg Gly Ala Phe Lys Asp Ser  
 915 920 925  
 Asn Thr Leu Ala Asn Val Thr Val Leu Gly Val Pro Ala Ala Pro Ser





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740			745			750									
Met	Arg	Ala	Leu	Ala	Trp	Glu	Phe	Pro	Asn	Asp	Pro	Ser	Leu	Ala	Ala
	755						760						765		
Val	Asp	Arg	Gln	Phe	Leu	Leu	Gly	Pro	Ser	Leu	Met	Val	Val	Pro	Val
	770						775						780		
Leu	Glu	Pro	Gln	Val	Asp	Thr	Val	Lys	Gly	Val	Phe	Pro	Gly	Val	Ala
	785				790						795				800
Gln	Gly	Gln	Val	Trp	Tyr	Asp	Trp	Tyr	Thr	Gln	Thr	Ala	Phe	Asp	Ala
				805						810					815
Gln	Pro	Gly	Val	Asn	Thr	Thr	Ile	Ser	Ala	Pro	Leu	Gly	His	Ile	Pro
				820						825					830
Val	Phe	Val	Arg	Gly	Gly	Ser	Val	Leu	Pro	Met	Gln	Gln	Pro	Ala	Leu
				835											845
Val	Thr	Arg	Asp	Val	Arg	Asn	Ser	Pro	Trp	Ser	Leu	Leu	Val	Ala	Leu
															850
Gly	Ser	Asp	Gly	Thr	Ala	Ser	Gly	Gln	Leu	Tyr	Val	Asp	Asp	Gly	Glu
					870										880
Ser	Ile	Thr	Pro	Pro	Ala	Ser	Leu	His	Val	Asp	Phe	Val	Ala	Ala	Asn
					885										895
Phe	Ser	Thr	Leu	Phe	Ala	Thr	Ala	Arg	Gly	Ala	Phe	Lys	Asp	Ser	Asn
					900										910
Thr	Leu	Ala	Asn	Val	Thr	Val	Leu	Gly	Val	Pro	Ala	Ala	Pro	Ser	Ser
															915
Ala	Val	Thr	Trp	Asn	Asn	Glu	Thr	Val	Pro	Ser	Glu	Ser	Val	Ser	Tyr
															930
Asn	Ala	Thr	Ser	Lys	Val	Leu	Val	Val	Asn	Gly	Leu	Gln	Ser	Leu	Thr
					945										950
Arg	Asp	Gly	Ala	Trp	Ser	Ser	Asp	Trp	Val	Leu	Lys	Trp			
					965										970

<210> SEQ ID NO 19  
 <211> LENGTH: 1815  
 <212> TYPE: DNA  
 <213> ORGANISM: Bifidobacterium longum

<400> SEQUENCE: 19

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atgaccgcaa ataatctcaa tgacgactgg tggaagcagg ccgtcgtcta ccagatctac    60
ccgcgcagct tcaaggagct taacggcgac ggactcggcg acatcgcggc cgtcaccgag    120
aagatggact acctgaagaa cctcggcgct gacgcaatct ggctctcccc gttctacccc    180
tccgatctgg cggacggcgg ctacgacgta atcgactacc gcaacgtcga cccgcgctta    240
ggcaccatgg aagacttcga cgcgatggcc aaggccgcgc acgaggccgg catcaaggtg    300
atcgtggaca tcgtgcccaa ccacaccgct gacaagcagc tgttcttcca ggaggccctc    360
gccgcccagc ctggctcccc ggcgcgcgac cgctacatct tccgcgacgg ccgcgggcgag    420
catggcgaac tgccccgaa cgactggcaa tccttcttcg gcggcccggc ctgggcgcgc    480
gtggccgacg gccagtggta tctgcacctg ttcgataagg cgcagccgga cgtcaactgg    540
aagaatccgg acatccacga ggagttcaag aagaccctgc gtttctggtc cgaccacggc    600
accgacggct tccgcatcga cgtggcgcac ggcctggcca aggacctcga atccaagccg    660
ttggaagagc tcggccgaga atacagcgtg gtcggcgtgc tgaatcatga cttcagccac    720
ccgctgttcg accgtcgcga ggtgcacgat atctaccgag aatggcgcaa ggtgttcaac    780
gagtacgaac cgccgcgctt tgccgtggcc gaggcgtggg tggcgcccga gcaccagcac    840
    
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ctgtacgctt cgatggaaga gctgggccag tccttcaact tcgacttgcg gcaagccaac   900
tggatgcccg acgagttccg cgaggccatc gccgcccggac tcaaggcggc ggccgaaacc   960
ggcggttcca ccaccacgtg ggtcatgaac aatcacgacg tgcccgcgag cccctcccgt   1020
tacggcctgc cgcagatcaa gggcgcaccc taccaccagc tgcccgcgca ctggctgctg   1080
cgcaacggca ctacctatcc cgaggatcgc gagcttgcca ctgcgccgcg ccgcgccgcc   1140
gctttgatgg agctcggcct gcccgggccc gcctatatct atcagggcga ggagctgggc   1200
ctgtttgagg tggccgatat tccgtgggat cacttgaggg atccgaccgc tttccacct   1260
gctcaggcca cgatggaaa gggcccgcac ggctgccgcg tgcccgtgcc gtggaccgcc   1320
gccgatgagc cggccttggc cgatttcagc cgtccgactc cggccgatga cggtaaccgc   1380
gagaaccatg tgcccgtgtg cgcgcggcgc cagttcgcca cgggcgcttc cttcgcttc   1440
tcgcccggta cgcgcgctga gggcgtgacg ccggccgccc acccgcacct gccgcaaccg   1500
ctgtggttca aggattacgc ggtggacgtg gagcaagccg acccggactc gatgctcgcg   1560
ctgtatcgcg ccgcaactgc gattcgccag gagtcgtga ccgccaccgc cgacaccacg   1620
gccgagcagg tggacatggg cgacgatgtg gtggcgtaca cccgcgcggc ggttggcggg   1680
cgggtgttca cctcaatcac caacttcggc aatgctccgg tcgcgctgcc cgatggctcc   1740
gtggtgctgg cgtccggccc gctgaccccc gaagcccagc tccccaccga cacttctgcg   1800
tgggttgttc agtag                                     1815

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&lt;210&gt; SEQ ID NO 20

&lt;211&gt; LENGTH: 604

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Bifidobacterium longum

&lt;400&gt; SEQUENCE: 20

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Met Thr Ala Asn Asn Leu Asn Asp Asp Trp Trp Lys Gln Ala Val Val
 1          5          10          15
Tyr Gln Ile Tyr Pro Arg Ser Phe Lys Asp Val Asn Gly Asp Gly Leu
          20          25          30
Gly Asp Ile Ala Gly Val Thr Glu Lys Met Asp Tyr Leu Lys Asn Leu
          35          40          45
Gly Val Asp Ala Ile Trp Leu Ser Pro Phe Tyr Pro Ser Asp Leu Ala
          50          55          60
Asp Gly Gly Tyr Asp Val Ile Asp Tyr Arg Asn Val Asp Pro Arg Leu
 65          70          75          80
Gly Thr Met Glu Asp Phe Asp Ala Met Ala Lys Ala Ala His Glu Ala
          85          90          95
Gly Ile Lys Val Ile Val Asp Ile Val Pro Asn His Thr Ala Asp Lys
          100          105          110
His Val Phe Phe Gln Glu Ala Leu Ala Ala Glu Pro Gly Ser Pro Ala
          115          120          125
Arg Asp Arg Tyr Ile Phe Arg Asp Gly Arg Gly Glu His Gly Glu Leu
          130          135          140
Pro Pro Asn Asp Trp Gln Ser Phe Phe Gly Gly Pro Ala Trp Ala Arg
          145          150          155          160
Val Ala Asp Gly Gln Trp Tyr Leu His Leu Phe Asp Lys Ala Gln Pro
          165          170          175
Asp Val Asn Trp Lys Asn Pro Asp Ile His Glu Glu Phe Lys Lys Thr
          180          185          190

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Leu Arg Phe Trp Ser Asp His Gly Thr Asp Gly Phe Arg Ile Asp Val  
 195 200 205

Ala His Gly Leu Ala Lys Asp Leu Glu Ser Lys Pro Leu Glu Glu Leu  
 210 215 220

Gly Arg Glu Tyr Ser Val Val Gly Val Leu Asn His Asp Phe Ser His  
 225 230 235 240

Pro Leu Phe Asp Arg Arg Glu Val His Asp Ile Tyr Arg Glu Trp Arg  
 245 250 255

Lys Val Phe Asn Glu Tyr Asp Pro Pro Arg Phe Ala Val Ala Glu Ala  
 260 265 270

Trp Val Val Pro Glu His Gln His Leu Tyr Ala Ser Met Asp Glu Leu  
 275 280 285

Gly Gln Ser Phe Asn Phe Asp Phe Ala Gln Ala Asn Trp Tyr Ala Asp  
 290 295 300

Glu Phe Arg Glu Ala Ile Ala Ala Gly Leu Lys Ala Ala Ala Glu Thr  
 305 310 315 320

Gly Gly Ser Thr Thr Thr Trp Val Met Asn Asn His Asp Val Pro Arg  
 325 330 335

Ser Pro Ser Arg Tyr Gly Leu Pro Gln Ile Lys Gly Ala Pro Tyr His  
 340 345 350

Gln Leu Pro His Asp Trp Leu Leu Arg Asn Gly Thr Thr Tyr Pro Glu  
 355 360 365

Asp Arg Glu Leu Gly Thr Arg Arg Ala Arg Ala Ala Ala Leu Met Glu  
 370 375 380

Leu Gly Leu Pro Gly Ala Ala Tyr Ile Tyr Gln Gly Glu Glu Leu Gly  
 385 390 395 400

Leu Phe Glu Val Ala Asp Ile Pro Trp Asp His Leu Glu Asp Pro Thr  
 405 410 415

Ala Phe His Thr Ala Gln Ala Thr Met Asp Lys Gly Arg Asp Gly Cys  
 420 425 430

Arg Val Pro Leu Pro Trp Thr Ala Ala Asp Glu Pro Ala Leu Ala Asp  
 435 440 445

Phe Ser Arg Pro Thr Pro Ala Asp Asp Gly Thr Gly Glu Asn His Val  
 450 455 460

Pro Leu Cys Ala Ala Gly Gln Phe Gly Thr Gly Ala Ser Phe Gly Phe  
 465 470 475 480

Ser Pro Ala Thr Arg Ala Glu Gly Val Thr Pro Ala Ala Asp Pro His  
 485 490 495

Leu Pro Gln Pro Leu Trp Phe Lys Asp Tyr Ala Val Asp Val Glu Gln  
 500 505 510

Ala Asp Pro Asp Ser Met Leu Ala Leu Tyr Arg Ala Ala Leu Ala Ile  
 515 520 525

Arg Gln Glu Ser Leu Thr Ala Thr Arg Asp Thr Thr Ala Glu Gln Val  
 530 535 540

Asp Met Gly Asp Asp Val Val Ala Tyr Thr Arg Ala Ala Val Gly Gly  
 545 550 555 560

Arg Val Phe Thr Ser Ile Thr Asn Phe Gly Asn Ala Pro Val Ala Leu  
 565 570 575

Pro Asp Gly Ser Val Val Leu Ala Ser Gly Pro Leu Thr Pro Glu Ala  
 580 585 590

Gln Leu Pro Thr Asp Thr Ser Ala Trp Val Val Gln  
 595 600

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<210> SEQ ID NO 21
<211> LENGTH: 1812
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: BloGlul_codon-optimized

<400> SEQUENCE: 21
gtgacagcaa ataatcttaa cgatgattgg tggaaacaag cagtggttta ccaaactac   60
ccgagatcat ttaaagacgt taatggcgac ggactgggag atattgctgg cgtgacagag   120
aaaatggact atctgaagaa tctgggagtg gatgctgatt ggctgtcacc gttctatccg   180
agcgatctgg ctgacggagg ctacgatgtc attgattata gaaatgtgga tccgagactt   240
ggcacgatgg aggatttoga cgcgatggcg aaggcagccc atgaagcagg cattaaggtt   300
attgtcgata ttgttccgaa tcatacggcg gacaacaatg tgttttttca agaggcactt   360
gccgctgaac ctggaagccc ggctagagat agatacattt tcagagatgg cagaggcgaa   420
cacggcgagc ttcctcctaa cgactggcaa tcattcttcg gaggccctgc atgggcaaga   480
gtggcggacg gccaatggta cctgcactct tttgataaag cacagccgga tgttaattgg   540
aaaaatcctg atattcacga agagttaaag aaaacactta gattctggtc agatcatggc   600
acggacggat ttagaattga tgtcgcacac ggccttgoga aagatctgga gtcaaaaaccg   660
cttgaggagc ttggaagaga atatagcgtg gttggagttc tgaatcatga cttcagccac   720
cctctgtttg acagaagaga ggttcatgac atttatagag aatggagaaa agttttcaat   780
gaatatgatc cgccgagatt tgcggttgcg gaggcctggg ttgtgcctga gcatcaacat   840
ctgtacgctt caatggacga gcttggccag tcattcaact ttgattttgc ccaagcaaat   900
tggatgacag atgaatttag agaggctatt gctgctggcc tgaagcagc ggctgaaacg   960
ggaggatcaa cgacaacatg ggttatgaat aatcacgatg ttcctagatc accgtcaaga   1020
tatggcctgc ctcagattaa gggcgcaccg tatcaaccagc ttcgcacga ttggctgctt   1080
agaaacggca cgacgtaccc ggaagataga gagctgggaa caagaagagc aagagcagca   1140
gctctgatgg aactgggact gcttgccgca gcatacatct atcaaggcga agaacttgga   1200
cttttcgaag ttgcagacat tccgtgggat catctggaag atcctacagc atttcacaca   1260
gcccaggcga caatggataa gggcagagac ggatgtagag tcccgtgccc gtggacagct   1320
gccgatgagc ctgcactggc agacttctca agaccgacac cggccgacga cggaacaggc   1380
gagaaccatg tgcgctttg tgcagccggc cagtttgcca caggcgctag ctttggattt   1440
tcaccggcga cgagagcggg aggcgtcaca cctgctgcag atcctcaact gccgcaacct   1500
ctgtggttca aggattatgc tgttgacggt gagcaagcag acccgactc aatgcttgca   1560
ctgtatagag cggccctggc tattagacaa gaaagcctta cggcaacgag agacacgaca   1620
gcgagacaag ttgatatggg cgatgatggt gttgcttata caagagccc agttggcgga   1680
agagtcttta cgtcaattac aaattttggc aatgcaccgg ttgcacttcc ggatggctca   1740
gttgttctgg catcaggccc gcttacacct gaagcacaac tgectacaga tacgtcagca   1800
tgggtcgttc aa                                     1812

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<210> SEQ ID NO 22
<211> LENGTH: 604
<212> TYPE: PRT
<213> ORGANISM: Bifidobacterium longum

<400> SEQUENCE: 22

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Met Thr Ala Asn Asn Leu Asn Asp Asp Trp Trp Lys Gln Ala Val Val  
 1 5 10 15  
 Tyr Gln Ile Tyr Pro Arg Ser Phe Lys Asp Val Asn Gly Asp Gly Leu  
 20 25 30  
 Gly Asp Ile Ala Gly Val Thr Glu Lys Met Asp Tyr Leu Lys Asn Leu  
 35 40 45  
 Gly Val Asp Ala Ile Trp Leu Ser Pro Phe Tyr Pro Ser Asp Leu Ala  
 50 55 60  
 Asp Gly Gly Tyr Asp Val Ile Asp Tyr Arg Asn Val Asp Pro Arg Leu  
 65 70 75 80  
 Gly Thr Met Glu Asp Phe Asp Ala Met Ala Lys Ala Ala His Glu Ala  
 85 90 95  
 Gly Ile Lys Val Ile Val Asp Ile Val Pro Asn His Thr Ala Asp Lys  
 100 105 110  
 His Val Phe Phe Gln Glu Ala Leu Ala Ala Glu Pro Gly Ser Pro Ala  
 115 120 125  
 Arg Asp Arg Tyr Ile Phe Arg Asp Gly Arg Gly Glu His Gly Glu Leu  
 130 135 140  
 Pro Pro Asn Asp Trp Gln Ser Phe Phe Gly Gly Pro Ala Trp Ala Arg  
 145 150 155 160  
 Val Ala Asp Gly Gln Trp Tyr Leu His Leu Phe Asp Lys Ala Gln Pro  
 165 170 175  
 Asp Val Asn Trp Lys Asn Pro Asp Ile His Glu Glu Phe Lys Lys Thr  
 180 185 190  
 Leu Arg Phe Trp Ser Asp His Gly Thr Asp Gly Phe Arg Ile Asp Val  
 195 200 205  
 Ala His Gly Leu Ala Lys Asp Leu Glu Ser Lys Pro Leu Glu Glu Leu  
 210 215 220  
 Gly Arg Glu Tyr Ser Val Val Gly Val Leu Asn His Asp Phe Ser His  
 225 230 235 240  
 Pro Leu Phe Asp Arg Arg Glu Val His Asp Ile Tyr Arg Glu Trp Arg  
 245 250 255  
 Lys Val Phe Asn Glu Tyr Asp Pro Pro Arg Phe Ala Val Ala Glu Ala  
 260 265 270  
 Trp Val Val Pro Glu His Gln His Leu Tyr Ala Ser Met Asp Glu Leu  
 275 280 285  
 Gly Gln Ser Phe Asn Phe Asp Phe Ala Gln Ala Asn Trp Tyr Ala Asp  
 290 295 300  
 Glu Phe Arg Glu Ala Ile Ala Ala Gly Leu Lys Ala Ala Ala Glu Thr  
 305 310 315 320  
 Gly Gly Ser Thr Thr Thr Trp Val Met Asn Asn His Asp Val Pro Arg  
 325 330 335  
 Ser Pro Ser Arg Tyr Gly Leu Pro Gln Ile Lys Gly Ala Pro Tyr His  
 340 345 350  
 Gln Leu Pro His Asp Trp Leu Leu Arg Asn Gly Thr Thr Tyr Pro Glu  
 355 360 365  
 Asp Arg Glu Leu Gly Thr Arg Arg Ala Arg Ala Ala Leu Met Glu  
 370 375 380  
 Leu Gly Leu Pro Gly Ala Ala Tyr Ile Tyr Gln Gly Glu Glu Leu Gly  
 385 390 395 400  
 Leu Phe Glu Val Ala Asp Ile Pro Trp Asp His Leu Glu Asp Pro Thr  
 405 410 415  
 Ala Phe His Thr Ala Gln Ala Thr Met Asp Lys Gly Arg Asp Gly Cys

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420				425				430							
Arg	Val	Pro	Leu	Pro	Trp	Thr	Ala	Ala	Asp	Glu	Pro	Ala	Leu	Ala	Asp
	435						440					445			
Phe	Ser	Arg	Pro	Thr	Pro	Ala	Asp	Asp	Gly	Thr	Gly	Glu	Asn	His	Val
	450					455					460				
Pro	Leu	Cys	Ala	Ala	Gly	Gln	Phe	Gly	Thr	Gly	Ala	Ser	Phe	Gly	Phe
	465				470					475					480
Ser	Pro	Ala	Thr	Arg	Ala	Glu	Gly	Val	Thr	Pro	Ala	Ala	Asp	Pro	His
			485						490					495	
Leu	Pro	Gln	Pro	Leu	Trp	Phe	Lys	Asp	Tyr	Ala	Val	Asp	Val	Glu	Gln
			500						505					510	
Ala	Asp	Pro	Asp	Ser	Met	Leu	Ala	Leu	Tyr	Arg	Ala	Ala	Leu	Ala	Ile
		515					520					525			
Arg	Gln	Glu	Ser	Leu	Thr	Ala	Thr	Arg	Asp	Thr	Thr	Ala	Glu	Gln	Val
	530					535									540
Asp	Met	Gly	Asp	Asp	Val	Val	Ala	Tyr	Thr	Arg	Ala	Ala	Val	Gly	Gly
	545				550					555					560
Arg	Val	Phe	Thr	Ser	Ile	Thr	Asn	Phe	Gly	Asn	Ala	Pro	Val	Ala	Leu
			565						570						575
Pro	Asp	Gly	Ser	Val	Val	Leu	Ala	Ser	Gly	Pro	Leu	Thr	Pro	Glu	Ala
			580						585					590	
Gln	Leu	Pro	Thr	Asp	Thr	Ser	Ala	Trp	Val	Val	Gln				
		595					600								

<210> SEQ ID NO 23  
 <211> LENGTH: 1812  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: BloGlu2\_codon-optimized

<400> SEQUENCE: 23

```

gtgacggcaa acaatctgaa tgatgattgg tggaaacaag cagtggctca tcagatttat    60
cctagatcat ttaaggatgt taatggcgac ggcctgggag atattgcagg cgtgacggaa    120
aaaatggatt atctgaagaa tctgggcggt gacgccattt ggctgtcacc gttttacccg    180
agcgacctgg ccgatggcgg ctatgacgtg attgattata gaaatgttga cccgagactg    240
ggcacgatgg acgattttga cgcaatggct gaagccgcac acgaggctgg cattaagtt    300
attgttgata ttgtcccga cccacacagca gacaagcacy tttttttcaa agaagcgctg    360
gcaagcgaac ctggctcacc ggcgagagac agatacattt ttagagacgg aagaggagag    420
catggcgaac tgccctcgaa cgattggcaa tcattctttg gcggacctgc ttgggctaga    480
gtcccggacg gccaatggta ccttcacctg ttcgataaag ctcagccgga tgtgaattgg    540
aaaaatcctg atatccatga agagttaag aagacgctta gattttggtc agatcatgga    600
acggatggat tcagaattga tgttgcacac ggacttgcaa aggatcttga atcaaaacct    660
cttgaagagc ttggaagaga atactcagtc gttggcgctt ttaatcacga cttttcacac    720
ccgctttttg acagaagaga ggtgcatgat atttacagag aatggagaaa agttttcaat    780
gaatatacac cgccgagatt cgcagtcgcc gaggcgtggg tgggtgcctga acatcagcat    840
ctttatgcat caatggatga acttggccag tcatttaact tcgatttcgc tcaagcaaat    900
tggtagcgtg atgaatttag aaaagcaatc gctgcgggcc tgaagcagc ggcagaaaca    960
ggaggaagca cgacaacatg ggtcatgaac aatcatgacg tccttagatc accgtcaaga   1020
    
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tacggccttc ctcaggtaa aggcgcaccg taccaccaac ttccgcacga ctggctgctg 1080
agagatggca cgacgtatcc ggaggataga gaactgggaa caagaagagc aagagcggct 1140
gccctgatgg agctgggact gcctggagca gcctatatct atcaaggcga ggaacttga 1200
ctgtttgagg ttgctgacat tccttgggat agactggaag atccgacagc gtttcataca 1260
gctcaggcga caatggacaa gggcagagat ggatgcagag ttccgcttcc gtggacggcg 1320
tcagacgaac cggcacttgc agacttctca agacctgccc ctgcagacga cggcacaggc 1380
gagaatcatg ttcctctgtg cgctgctgga caattcgaa caggeccttc attcgcttt 1440
agccctgccg ttagagcaga cggagttaca cctgcggccg acccgcatct gcctcaaccg 1500
ctttggttta aagattatgc agtggatgtc gagcaagcag atccggactc aatgtatacg 1560
ctgtatcagc ctgcactggc aattagacaa gaatcactga cagctacaag agatacaaca 1620
gctgagcagg tggacatggg agcagatgtg gttgcatata gaagagccgc ggtcgaagga 1680
agaacattta cgtcagttac gaactttggc acagctccgg tttcactgcc ggaaggctca 1740
gtggtgctga cgagcggacc gcttacgct gacggacagc tgccgacaga cacaagcgca 1800
tgggttatta ag 1812

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&lt;210&gt; SEQ ID NO 24

&lt;211&gt; LENGTH: 604

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Bifidobacterium longum

&lt;400&gt; SEQUENCE: 24

```

Met Thr Ala Asn Asn Leu Asn Asp Asp Trp Trp Lys Gln Ala Val Val
1          5          10          15
Tyr Gln Ile Tyr Pro Arg Ser Phe Lys Asp Val Asn Gly Asp Gly Leu
20          25          30
Gly Asp Ile Ala Gly Val Thr Glu Lys Met Asp Tyr Leu Lys Asn Leu
35          40          45
Gly Val Asp Ala Ile Trp Leu Ser Pro Phe Tyr Pro Ser Asp Leu Ala
50          55          60
Asp Gly Gly Tyr Asp Val Ile Asp Tyr Arg Asn Val Asp Pro Arg Leu
65          70          75          80
Gly Thr Met Asp Asp Phe Asp Ala Met Ala Glu Ala Ala His Glu Ala
85          90          95
Gly Ile Lys Val Ile Val Asp Ile Val Pro Asn His Thr Ala Asp Lys
100         105         110
His Val Phe Phe Lys Glu Ala Leu Ala Ser Glu Pro Gly Ser Pro Ala
115         120         125
Arg Asp Arg Tyr Ile Phe Arg Asp Gly Arg Gly Glu His Gly Glu Leu
130         135         140
Pro Pro Asn Asp Trp Gln Ser Phe Phe Gly Gly Pro Ala Trp Ala Arg
145         150         155         160
Val Pro Asp Gly Gln Trp Tyr Leu His Leu Phe Asp Lys Ala Gln Pro
165         170         175
Asp Val Asn Trp Lys Asn Pro Asp Ile His Glu Glu Phe Lys Lys Thr
180         185         190
Leu Arg Phe Trp Ser Asp His Gly Thr Asp Gly Phe Arg Ile Asp Val
195         200         205
Ala His Gly Leu Ala Lys Asp Leu Glu Ser Lys Pro Leu Glu Glu Leu
210         215         220
Gly Arg Glu Tyr Ser Val Val Gly Val Leu Asn His Asp Phe Ser His

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225		230		235		240									
Pro	Leu	Phe	Asp	Arg	Arg	Glu	Val	His	Asp	Ile	Tyr	Arg	Glu	Trp	Arg
			245						250					255	
Lys	Val	Phe	Asn	Glu	Tyr	Thr	Pro	Pro	Arg	Phe	Ala	Val	Ala	Glu	Ala
			260						265					270	
Trp	Val	Val	Pro	Glu	His	Gln	His	Leu	Tyr	Ala	Ser	Met	Asp	Glu	Leu
			275					280					285		
Gly	Gln	Ser	Phe	Asn	Phe	Asp	Phe	Ala	Gln	Ala	Asn	Trp	Tyr	Ala	Asp
			290			295					300				
Glu	Phe	Arg	Lys	Ala	Ile	Ala	Ala	Gly	Leu	Lys	Ala	Ala	Ala	Glu	Thr
			305			310					315				320
Gly	Gly	Ser	Thr	Thr	Thr	Trp	Val	Met	Asn	Asn	His	Asp	Val	Pro	Arg
					325					330					335
Ser	Pro	Ser	Arg	Tyr	Gly	Leu	Pro	Gln	Val	Lys	Gly	Ala	Pro	Tyr	His
			340						345					350	
Gln	Leu	Pro	His	Asp	Trp	Leu	Leu	Arg	Asp	Gly	Thr	Thr	Tyr	Pro	Glu
			355					360						365	
Asp	Arg	Glu	Leu	Gly	Thr	Arg	Arg	Ala	Arg	Ala	Ala	Ala	Leu	Met	Glu
			370			375							380		
Leu	Gly	Leu	Pro	Gly	Ala	Ala	Tyr	Ile	Tyr	Gln	Gly	Glu	Glu	Leu	Gly
			385			390				395					400
Leu	Phe	Glu	Val	Ala	Asp	Ile	Pro	Trp	Asp	Arg	Leu	Glu	Asp	Pro	Thr
				405					410						415
Ala	Phe	His	Thr	Ala	Gln	Ala	Thr	Met	Asp	Lys	Gly	Arg	Asp	Gly	Cys
			420						425					430	
Arg	Val	Pro	Leu	Pro	Trp	Thr	Ala	Ser	Asp	Glu	Pro	Ala	Leu	Ala	Asp
			435				440							445	
Phe	Ser	Arg	Pro	Ala	Pro	Ala	Asp	Asp	Gly	Thr	Gly	Glu	Asn	His	Val
			450				455					460			
Pro	Leu	Cys	Ala	Ala	Gly	Gln	Phe	Gly	Thr	Gly	Ala	Ser	Phe	Gly	Phe
			465			470				475					480
Ser	Pro	Ala	Val	Arg	Ala	Asp	Gly	Val	Thr	Pro	Ala	Ala	Asp	Pro	His
				485					490						495
Leu	Pro	Gln	Pro	Leu	Trp	Phe	Lys	Asp	Tyr	Ala	Val	Asp	Val	Glu	Gln
			500					505						510	
Ala	Asp	Pro	Asp	Ser	Met	Tyr	Thr	Leu	Tyr	His	Ala	Ala	Leu	Ala	Ile
			515					520						525	
Arg	Gln	Glu	Ser	Leu	Thr	Ala	Thr	Arg	Asp	Thr	Thr	Ala	Glu	Gln	Val
			530				535							540	
Asp	Met	Gly	Ala	Asp	Val	Val	Ala	Tyr	Arg	Arg	Ala	Ala	Val	Glu	Gly
			545			550				555					560
Arg	Thr	Phe	Thr	Ser	Val	Thr	Asn	Phe	Gly	Thr	Ala	Pro	Val	Ser	Leu
				565					570						575
Pro	Glu	Gly	Ser	Val	Val	Leu	Thr	Ser	Gly	Pro	Leu	Thr	Pro	Asp	Gly
				580					585					590	
Gln	Leu	Pro	Thr	Asp	Thr	Ser	Ala	Trp	Val	Ile	Lys				
			595					600							

&lt;210&gt; SEQ ID NO 25

&lt;211&gt; LENGTH: 1815

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Bifidobacterium longum

&lt;400&gt; SEQUENCE: 25

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atgaccgcca acaacctcaa tgacgactgg tggaaagcagg ccgtcgtcta ccagatctac   60
ccgcgcagct tcaaggagct caacggcgac ggactcggcg acatcgcggg cgtcaccgag   120
aagatggatt acctgaagaa cctcggcgtc gacgcgatct ggctctcccc gttctacccc   180
tcggatctgg cggacggcgg ctacgaactg atcgactacc gcaacgtcga cccgcgcta   240
ggcaccatgg acgacttcga cgcgatggcc gaggcgcgc acgagggcgg catcaaagtg   300
atcgtggaca tcgtgcccaa ccacaccgcc gacaagcagc tgtttctcaa ggaagccctc   360
gcctccgaac ctggctcccc cgcacgcgat cgctatatct tccgtgacgg ccgcgcgag   420
cacggcgaac tgccgccgaa cgactggcag tccttcttcg gcggcccggc ctgggcgcgc   480
gtgcccgaag gccagtggta cctgcactcg ttcgacaagg cgcagccgga cgtcaactgg   540
aagaatccgg acatccacga ggagttcaaa aagaccctgc gcttctggtc cgaccacggc   600
accgacggct tccgcatcga cgtagcgcac ggcctggcca aagaccctga atccaagccg   660
ctggaggagc tcggccgcga atacagcgtg gtcggcgtgc tgaaccacga cttcagccac   720
ccgctgttcg accgccgcga ggtgcacgac atctaaccgc aatggcgcaa ggtgttcaac   780
gaatacactc cgccgcgctt cgccgtggcc gaggcgtggg tggtgcccga gcaccagcac   840
ctgtacgctt cgatggacga gctgggcccag tccttcaact tcgacttcgc gcaggccaac   900
tggtatgccc acgagttccg caaggccatc gccgcggggc tcaaggcggc ggccgaaacc   960
ggcggctcca ccaccacgtg ggtcatgaac aatcatgacg tgccgcgcag cccctcccgc  1020
tatgtctgct cgcaggtaaa gggcgcgcgc taccaccagc tgccacacga ctggctgctg  1080
cgcgacggca ccacctaccg ggagaaccgc gaactcggca cccgccgtgc ccgtgccgcc  1140
gcgctgatgg agctcggcct gcccggtgcc gcatacatct atcagggcga ggaactgggc  1200
ctgtttgagg tggccgatat tccgtgggat cacttgaggg atccgaccgc cttccacacc  1260
acccgcaaca cgatggacaa gggccgcgac ggctgccgcg tgccgctgcc gtggaccgcc  1320
gccgatgagc cggccttggc cgatttcagc cgtccggctc cggccgatga cggtaccggc  1380
gaaaaccatg tgccgctgtg cgccgcgggc cagttcggca cgggcgcttc cttcgcttc  1440
tcccctgcgg ttcgcgcgca tggcgtgacg ccggccgcgc acccgcaact gccgcagccg  1500
ctgtggttca aggattacgc ggtggacgtg gagcaagccg acccggaact gatgctcgcg  1560
ctgtatcgcg ccgcaactgg gattcgccag gagtcgtga ccgccacgcg cgacaccacg  1620
gccgagcagg tggacatggg cgacgatgtg gtggcgtaca cccgcgcggc ggttggcggg  1680
cgggtgttca cctcaatcac caacttcggc aatgccccgg tcgcgctgcc cgatggctcc  1740
gtggtgctgg cgtccggccc gctgaccccc gaaggccagc tccccaccga cacttctgcg  1800
tgggttatca agtag                                     1815

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&lt;210&gt; SEQ ID NO 26

&lt;211&gt; LENGTH: 604

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Bifidobacterium longum

&lt;400&gt; SEQUENCE: 26

```

Met Thr Ala Asn Asn Leu Asn Asp Asp Trp Trp Lys Gln Ala Val Val
1           5           10          15

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Tyr Gln Ile Tyr Pro Arg Ser Phe Lys Asp Val Asn Gly Asp Gly Leu
20           25           30

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Gly Asp Ile Ala Gly Val Thr Glu Lys Met Asp Tyr Leu Lys Asn Leu
35           40           45

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Gly Val Asp Ala Ile Trp Leu Ser Pro Phe Tyr Pro Ser Asp Leu Ala  
 50 55 60  
 Asp Gly Gly Tyr Asp Val Ile Asp Tyr Arg Asn Val Asp Pro Arg Leu  
 65 70 75 80  
 Gly Thr Met Asp Asp Phe Asp Ala Met Ala Glu Ala Ala His Glu Ala  
 85 90 95  
 Gly Ile Lys Val Ile Val Asp Ile Val Pro Asn His Thr Ala Asp Lys  
 100 105 110  
 His Val Phe Phe Lys Glu Ala Leu Ala Ser Glu Pro Gly Ser Pro Ala  
 115 120 125  
 Arg Asp Arg Tyr Ile Phe Arg Asp Gly Arg Gly Glu His Gly Glu Leu  
 130 135 140  
 Pro Pro Asn Asp Trp Gln Ser Phe Phe Gly Gly Pro Ala Trp Ala Arg  
 145 150 155 160  
 Val Pro Asp Gly Gln Trp Tyr Leu His Leu Phe Asp Lys Ala Gln Pro  
 165 170 175  
 Asp Val Asn Trp Lys Asn Pro Asp Ile His Glu Glu Phe Lys Lys Thr  
 180 185 190  
 Leu Arg Phe Trp Ser Asp His Gly Thr Asp Gly Phe Arg Ile Asp Val  
 195 200 205  
 Ala His Gly Leu Ala Lys Asp Leu Glu Ser Lys Pro Leu Glu Glu Leu  
 210 215 220  
 Gly Arg Glu Tyr Ser Val Val Gly Val Leu Asn His Asp Phe Ser His  
 225 230 235 240  
 Pro Leu Phe Asp Arg Arg Glu Val His Asp Ile Tyr Arg Glu Trp Arg  
 245 250 255  
 Lys Val Phe Asn Glu Tyr Thr Pro Pro Arg Phe Ala Val Ala Glu Ala  
 260 265 270  
 Trp Val Val Pro Glu His Gln His Leu Tyr Ala Ser Met Asp Glu Leu  
 275 280 285  
 Gly Gln Ser Phe Asn Phe Asp Phe Ala Gln Ala Asn Trp Tyr Ala Asp  
 290 295 300  
 Glu Phe Arg Lys Ala Ile Ala Ala Gly Leu Lys Ala Ala Ala Glu Thr  
 305 310 315 320  
 Gly Gly Ser Thr Thr Thr Trp Val Met Asn Asn His Asp Val Pro Arg  
 325 330 335  
 Ser Pro Ser Arg Tyr Gly Leu Pro Gln Val Lys Gly Ala Pro Tyr His  
 340 345 350  
 Gln Leu Pro His Asp Trp Leu Leu Arg Asp Gly Thr Thr Tyr Pro Glu  
 355 360 365  
 Asn Arg Glu Leu Gly Thr Arg Arg Ala Arg Ala Ala Ala Leu Met Glu  
 370 375 380  
 Leu Gly Leu Pro Gly Ala Ala Tyr Ile Tyr Gln Gly Glu Glu Leu Gly  
 385 390 395 400  
 Leu Phe Glu Val Ala Asp Ile Pro Trp Asp His Leu Glu Asp Pro Thr  
 405 410 415  
 Ala Phe His Thr Thr Arg Asn Thr Met Asp Lys Gly Arg Asp Gly Cys  
 420 425 430  
 Arg Val Pro Leu Pro Trp Thr Ala Ala Asp Glu Pro Ala Leu Ala Asp  
 435 440 445  
 Phe Ser Arg Pro Ala Pro Ala Asp Asp Gly Thr Gly Glu Asn His Val  
 450 455 460  
 Pro Leu Cys Ala Ala Gly Gln Phe Gly Thr Gly Ala Ser Phe Gly Phe

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465	470	475	480
Ser Pro Ala Val Arg Ala Asp Gly Val Thr Pro Ala Ala Asp Pro His	485	490	495
Leu Pro Gln Pro Leu Trp Phe Lys Asp Tyr Ala Val Asp Val Glu Gln	500	505	510
Ala Asp Pro Asp Ser Met Leu Ala Leu Tyr Arg Ala Ala Leu Ala Ile	515	520	525
Arg Gln Glu Ser Leu Thr Ala Thr Arg Asp Thr Thr Ala Glu Gln Val	530	535	540
Asp Met Gly Asp Asp Val Val Ala Tyr Thr Arg Ala Ala Val Gly Gly	545	550	555
Arg Val Phe Thr Ser Ile Thr Asn Phe Gly Asn Ala Pro Val Ala Leu	565	570	575
Pro Asp Gly Ser Val Val Leu Ala Ser Gly Pro Leu Thr Pro Glu Gly	580	585	590
Gln Leu Pro Thr Asp Thr Ser Ala Trp Val Ile Lys	595	600	

&lt;210&gt; SEQ ID NO 27

&lt;211&gt; LENGTH: 1812

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: BloGlu3\_codon-optimized

&lt;400&gt; SEQUENCE: 27

```

gtgacagcaa acaacctgaa tgacgactgg tggaaacagg ccgtcgtgta tcaaatttac    60
cctagatcat ttaaggacgt taatggcgat ggactgggag atattgcagg cgtcacggag    120
aagatggatt accttaaaaa cctgggcggt gatgctatgt ggctgtcacc gttttaccgg    180
tcagacctgg ctgatggcgg ctatgacggt atcgattata gaaatgtgga tcctagactg    240
ggcacaatgg atgattttga cgcaatggcc gaagccgcgc atgaagcggg aattaaagt    300
attgttgata tcgttccata tcatacagca gataagcatg tttttttcaa agaagcactg    360
gcctcagaac cgggctcacc tgcaagagat agatacattt ttagagacgg aagaggcgaa    420
cacggcgagc tgctccgaa tgactggcaa tcattttttg gaggacctgc atgggcaaga    480
gtccctgacg gacaatggta tctgcacctt ttcgataaag cacaaccgga tgttaattgg    540
aaaaaccctg acatccatga agaatttaag aaaacactga gattttggtc agatcacggc    600
acagacggat ttagaattga tgtggcacaat ggacttgcaa aagatctgga aagcaaaccg    660
ctggaggaac tgggaagaga gtacagcgtt gttggcggtc ttaatcatga cttttcacat    720
ccgctgtttg acagaagaga agtgcacgac atctacagag aatggagaaa ggtgtttaac    780
gaatatacac cgccgagatt tgcagtcgca gaagcatggg tgggtgcctga acaccaacat    840
ctgtatgcat caatggacga gctgggocaa tcattcaatt tcgactttgc acaagctaatt    900
tggatgacg atgagttcag aaaagctatc gctgcgggcc tgaaagcagc agctgagacg    960
ggcggatcaa cgacaacatg ggtcatgaat aatcacgatg ttccgagatc accgtcaaga   1020
tatggcctgc cgcagggttaa gggcgaccg tatcatcagc ttccgcacga ttggcttctg   1080
agagacggca cgagctaccc ggagaataga gagctgggaa cgagaagagc aagagcagcg   1140
gcgcttatgg aacttgact gctggagcgg gcttacattt atcagggcga ggagcttggc   1200
ctttttgagg tggcagatat tccttgggat catcttgaag atccgacagc atttcacaca   1260
acaagaaata cgatggataa aggcagagat ggctgcagag tgcctctgcc gtggacagct   1320

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gccgatgagc ctgcccttgc cgacttttagc agaccggcac ctgccgacga cggaacggga 1380
gagaaccaacg ttcctctgtg tgcagcgggc cagtttgcca cgggagcadc atttggatc 1440
tcaccggcgc ttagagccga tggcgttaca ccggctgcgg acccgcatct gcctcagccg 1500
ctgtgggtta aagattatgc agttgacgtt gaacaagccg atcctgattc aatgctggca 1560
ctgtatagag ctgcacttgc tattagacaa gaatcactta cggcaacgag agatacaacg 1620
gcggaacagg tcgacatggg cgatgatgtc gtcgcatata cgagagcggc cgtgggagga 1680
agagttttta caagcattac aaattttggc aacgcacctg tcgcaactgc ggatggcagc 1740
gtcgttctgg cttcaggacc tctgacaccg gagggacaac tgcctacaga cacgtcagct 1800
tgggttatta ag 1812

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&lt;210&gt; SEQ ID NO 28

&lt;211&gt; LENGTH: 585

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Bifidobacterium pseudolongum

&lt;400&gt; SEQUENCE: 28

```

Met Thr Leu Asn Asn Thr His Asp Asp Trp Trp Lys Gln Ala Val Val
1          5          10          15
Tyr Gln Val Tyr Pro Arg Ser Phe Arg Asp Ala Asn Gly Asp Gly Leu
20          25          30
Gly Asp Ile Ala Gly Ile Thr Ser Arg Ile Pro Tyr Leu Arg Gln Leu
35          40          45
Gly Val Asp Ala Leu Trp Leu Ser Pro Phe Tyr Pro Ser Glu Leu Ala
50          55          60
Asp Gly Gly Tyr Asp Val Ile Asp Tyr Arg Asn Val Asp Pro Arg Leu
65          70          75          80
Gly Thr Leu Asp Asp Phe Asp Ala Met Val Ala Ala Ala His Ser Ala
85          90          95
Gly Leu Lys Val Val Val Asp Ile Val Pro Asn His Thr Ser Asn Met
100         105         110
His Pro Trp Phe Gln Glu Ala Leu Ala Ser Ala Pro Gly Ser Pro Ala
115         120         125
Arg Asp Arg Tyr Ile Phe Arg Asp Gly Glu Gly Ala His Gly Glu Leu
130         135         140
Pro Pro Asn Asn Trp Gln Ser Leu Phe Gly Gly Pro Ala Trp Glu Ala
145         150         155         160
Ala Gly Asp Gly Gln Trp Tyr Leu His Leu Phe Thr Lys Glu Gln Pro
165         170         175
Asp Leu Asn Trp Lys Asn Pro Asp Val His Glu Asp Phe Arg Thr Thr
180         185         190
Leu Arg Phe Trp Ser Asp Arg Gly Val Asp Gly Phe Arg Ile Asp Val
195         200         205
Ala His Gly Leu Ala Lys Asp Leu Asp Ser Glu Pro Leu Lys Asp Leu
210         215         220
Glu Arg Phe Pro Val Gly Asn Pro Val Pro Gly His Pro Leu Trp
225         230         235         240
Asp Arg Pro Glu Val His Glu Ile Tyr Arg Glu Trp Asn Lys Val Phe
245         250         255
Asn Glu Tyr Asp Pro Pro Arg Phe Ala Val Gly Glu Ala Trp Val Pro
260         265         270
Ala Glu His Gln His Leu Tyr Ala Ser Lys Asp Glu Leu Gly Gln Val

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275					280					285					
Phe	Asn	Phe	Glu	Phe	Ala	Lys	Ala	Asn	Trp	Phe	Ala	Asp	Asp	Phe	Arg
290						295					300				
Leu	Ala	Ile	Glu	Glu	Gly	Leu	Ala	Ser	Ala	Asp	Glu	Ser	Lys	Ser	Thr
305					310					315					320
Thr	Thr	Trp	Val	Met	Ser	Asn	His	Asp	Val	Pro	Arg	His	Val	Ser	Arg
				325					330					335	
Tyr	Gly	Leu	Pro	Gln	Val	His	Thr	Arg	Gly	Tyr	His	Glu	Leu	Pro	Asn
			340					345						350	
Asp	Trp	Leu	Leu	Arg	Asn	Gly	Thr	Thr	Tyr	Ile	Glu	Asp	Arg	Glu	Leu
		355					360					365			
Gly	Thr	Arg	Arg	Ala	Arg	Ala	Ala	Ile	Leu	Met	Glu	Leu	Gly	Leu	Pro
370						375					380				
Gly	Ser	Val	Tyr	Val	Tyr	Gln	Gly	Glu	Glu	Leu	Gly	Leu	Pro	Glu	Val
385					390					395					400
Ala	Thr	Ile	Pro	Trp	Asp	His	Leu	Glu	Asp	Pro	Val	Ala	Phe	Asn	Thr
				405					410						415
Asp	His	Ser	Asp	Ala	Ala	Lys	Gly	Arg	Asp	Gly	Cys	Arg	Val	Pro	Leu
			420					425					430		
Pro	Trp	Ser	Ala	Gln	Asp	Met	Pro	Gln	Pro	Ala	Pro	Trp	Asp	Pro	Glu
		435					440						445		
Phe	Gly	Thr	Gly	Ala	Ser	Phe	Gly	Phe	Ser	Glu	His	Ala	Gly	Gly	Arg
450						455						460			
Ala	Ser	Ala	Asp	Pro	His	Leu	Pro	Gln	Pro	Leu	Trp	Tyr	Ala	Gly	Tyr
465					470					475					480
Ala	Ala	Asp	Met	Glu	Asp	Thr	Asp	Pro	Ala	Ser	Met	Leu	Asn	Leu	Tyr
			485					490							495
Arg	Arg	Ala	Met	His	Trp	Arg	Gln	Glu	His	Leu	Thr	Pro	Thr	Gly	Asp
			500					505						510	
Thr	Ser	Leu	Thr	Trp	Leu	Ser	Pro	Gln	Ser	Phe	Ala	Asp	Cys	Gly	Asp
		515					520						525		
Asp	Val	Val	Ala	Tyr	Ala	Arg	Pro	Leu	Ala	Asp	Asp	Ser	Gly	Asp	Arg
530						535						540			
Phe	Val	Cys	Ile	Val	Asn	Phe	Gly	Ala	Ala	Ser	Ile	Glu	Leu	Pro	His
545					550					555					560
Gly	Asp	Val	Met	Met	Arg	Ser	Ile	Pro	Phe	Asp	Gly	Tyr	Gln	Leu	Pro
				565					570						575
Ala	Asp	Ala	Ala	Val	Trp	Met	Arg	Ile							
			580					585							

&lt;210&gt; SEQ ID NO 29

&lt;211&gt; LENGTH: 1755

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: BpsGlu1\_codon-optimized

&lt;400&gt; SEQUENCE: 29

```

gtgacactga ataatacaca cgatgactgg tggaaacaag ccgttggtga tcaagtttat    60
ccgagatcat ttagagacgc taacggcgat ggactgggag acatcgcagg cattacgagc    120
agaatcccgt atcttagaca acttgcgctg gacgcgcttt ggctttcacc gttttatcct    180
agcgagctgg cagacggcgg atatgatggtt attgattaca gaaatgttga cccgagactt    240
ggcacactgg acgactttga tgctatggtg gcggctgccc atagcgcggg actgaaagtt    300

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gttgtgata tcgttccgaa tcatacaagc aacatgcacc cttggttcca agaggcgctg 360
gcgagcgcac cgggatcacc tgcgagagat agatacattt tcagagatgg agaaggcgca 420
cacggcgagc ttccgcctaa caattggcaa agcctgtttg gaggaccggc ctgggaagca 480
gcaggcgaoc gacaatggta cctgcacott ttacaaaag aacagcctga tctgaattgg 540
aaaaatcctg acgttcacga ggatttcaga acgacaccta gattctggtc agacagaggc 600
gttgatggct ttagaatoga cgttgacat ggacttgcca aagacctgga tagcgaaccg 660
ctgaaggatc ttgagagatt cccggtcoga ggaaatccgg ttccgggcca tccgctgtgg 720
gatagacctg aagtgcacga gatttcacaga gagtggata aagtccttaa tgaatacgac 780
ccgctagat tcgctgttg cgaagcatgg gtgectgccc aacatcaaca tctttatgcc 840
tcaaaggatg agctgggaca agtgttcaac ttcgaatttg ctaaggcaaa ttggtttgca 900
gatgacttta gactggcaat tgaggaggc ctggcctcag cggacgagtc aaaatcaacg 960
acaacatggg ttatgtcaaa tcacgacgtg ccgagacacg tgagcagata tggccttccg 1020
caagttcata cgagaggcta tcacgagctg cctaacgatt ggctgctgag aaatggcaca 1080
acatatattg aagatagaga actgggcaca agaagagcaa gagcccgcat cctgatggaa 1140
cttgacttc ctggatcagt ttatgtttat caaggcgagg agcttggcct gcctgaggtg 1200
gcaacaattc cttgggacca cctggaagat ccggtgcct tcaacacaga tcaactcagat 1260
gcagcaaaag gaagagacgg atgtagagtg ccgctgcccgt ggtcagctca ggatagccc 1320
cagcctgcgc cgtgggatcc ggaatttggc acgggcccac catttggatt ctcagagcat 1380
gccggaggca gagcttcagc agaccgcat ctgccgaac ctctgtggta tgcaggctat 1440
gctgccgata tggaggatac ggatcctcgc tcaatgctga atctgtatag aagagctatg 1500
cactggagac aggagcatct tacacctaca ggcgatacat cactgacatg gctgtcaccg 1560
caatcattcg cagattggcg agatgatgtc gtggcatacg caagaccgct ggctgacgat 1620
agcggcgaca gatttgtttg cattgttaac ttcggagcag cgagcatcga actgcctcac 1680
ggagacgtta tgatgagatc aatcccttcc gatggatacc agcttccctg ggacgctgcg 1740
gtgtggatga gaatt 1755

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&lt;210&gt; SEQ ID NO 30

&lt;211&gt; LENGTH: 585

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: BpsGlui protein\_encoded by optimized sequence

&lt;400&gt; SEQUENCE: 30

```

Met Thr Leu Asn Asn Thr His Asp Asp Trp Trp Lys Gln Ala Val Val
1           5           10          15
Tyr Gln Val Tyr Pro Arg Ser Phe Arg Asp Ala Asn Gly Asp Gly Leu
20          25          30
Gly Asp Ile Ala Gly Ile Thr Ser Arg Ile Pro Tyr Leu Arg Gln Leu
35          40          45
Gly Val Asp Ala Leu Trp Leu Ser Pro Phe Tyr Pro Ser Glu Leu Ala
50          55          60
Asp Gly Gly Tyr Asp Val Ile Asp Tyr Arg Asn Val Asp Pro Arg Leu
65          70          75          80
Gly Thr Leu Asp Asp Phe Asp Ala Met Val Ala Ala Ala His Ser Ala
85          90          95
Gly Leu Lys Val Val Val Asp Ile Val Pro Asn His Thr Ser Asn Met

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100				105				110							
His	Pro	Trp	Phe	Gln	Glu	Ala	Leu	Ala	Ser	Ala	Pro	Gly	Ser	Pro	Ala
	115						120						125		
Arg	Asp	Arg	Tyr	Ile	Phe	Arg	Asp	Gly	Glu	Gly	Ala	His	Gly	Glu	Leu
	130					135					140				
Pro	Pro	Asn	Asn	Trp	Gln	Ser	Leu	Phe	Gly	Gly	Pro	Ala	Trp	Glu	Ala
	145				150					155				160	
Ala	Gly	Asp	Gly	Gln	Trp	Tyr	Leu	His	Leu	Phe	Thr	Lys	Glu	Gln	Pro
				165						170				175	
Asp	Leu	Asn	Trp	Lys	Asn	Pro	Asp	Val	His	Glu	Asp	Phe	Arg	Thr	Thr
		180						185					190		
Leu	Arg	Phe	Trp	Ser	Asp	Arg	Gly	Val	Asp	Gly	Phe	Arg	Ile	Asp	Val
	195						200					205			
Ala	His	Gly	Leu	Ala	Lys	Asp	Leu	Asp	Ser	Glu	Pro	Leu	Lys	Asp	Leu
	210					215					220				
Glu	Arg	Phe	Pro	Val	Gly	Gly	Asn	Pro	Val	Pro	Gly	His	Pro	Leu	Trp
	225				230					235				240	
Asp	Arg	Pro	Glu	Val	His	Glu	Ile	Tyr	Arg	Glu	Trp	Asn	Lys	Val	Phe
				245					250					255	
Asn	Glu	Tyr	Asp	Pro	Pro	Arg	Phe	Ala	Val	Gly	Glu	Ala	Trp	Val	Pro
			260					265					270		
Ala	Glu	His	Gln	His	Leu	Tyr	Ala	Ser	Lys	Asp	Glu	Leu	Gly	Gln	Val
		275					280					285			
Phe	Asn	Phe	Glu	Phe	Ala	Lys	Ala	Asn	Trp	Phe	Ala	Asp	Asp	Phe	Arg
	290				295						300				
Leu	Ala	Ile	Glu	Glu	Gly	Leu	Ala	Ser	Ala	Asp	Glu	Ser	Lys	Ser	Thr
	305				310					315				320	
Thr	Thr	Trp	Val	Met	Ser	Asn	His	Asp	Val	Pro	Arg	His	Val	Ser	Arg
				325					330					335	
Tyr	Gly	Leu	Pro	Gln	Val	His	Thr	Arg	Gly	Tyr	His	Glu	Leu	Pro	Asn
		340						345					350		
Asp	Trp	Leu	Leu	Arg	Asn	Gly	Thr	Thr	Tyr	Ile	Glu	Asp	Arg	Glu	Leu
		355					360					365			
Gly	Thr	Arg	Arg	Ala	Arg	Ala	Ala	Ile	Leu	Met	Glu	Leu	Gly	Leu	Pro
	370				375						380				
Gly	Ser	Val	Tyr	Val	Tyr	Gln	Gly	Glu	Glu	Leu	Gly	Leu	Pro	Glu	Val
	385				390					395				400	
Ala	Thr	Ile	Pro	Trp	Asp	His	Leu	Glu	Asp	Pro	Val	Ala	Phe	Asn	Thr
				405					410					415	
Asp	His	Ser	Asp	Ala	Ala	Lys	Gly	Arg	Asp	Gly	Cys	Arg	Val	Pro	Leu
			420					425					430		
Pro	Trp	Ser	Ala	Gln	Asp	Met	Pro	Gln	Pro	Ala	Pro	Trp	Asp	Pro	Glu
		435					440						445		
Phe	Gly	Thr	Gly	Ala	Ser	Phe	Gly	Phe	Ser	Glu	His	Ala	Gly	Gly	Arg
	450					455					460				
Ala	Ser	Ala	Asp	Pro	His	Leu	Pro	Gln	Pro	Leu	Trp	Tyr	Ala	Gly	Tyr
	465				470					475				480	
Ala	Ala	Asp	Met	Glu	Asp	Thr	Asp	Pro	Ala	Ser	Met	Leu	Asn	Leu	Tyr
				485					490					495	
Arg	Arg	Ala	Met	His	Trp	Arg	Gln	Glu	His	Leu	Thr	Pro	Thr	Gly	Asp
			500						505					510	
Thr	Ser	Leu	Thr	Trp	Leu	Ser	Pro	Gln	Ser	Phe	Ala	Asp	Cys	Gly	Asp
		515					520						525		

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Asp Val Val Ala Tyr Ala Arg Pro Leu Ala Asp Asp Ser Gly Asp Arg  
 530 535 540

Phe Val Cys Ile Val Asn Phe Gly Ala Ala Ser Ile Glu Leu Pro His  
 545 550 555 560

Gly Asp Val Met Met Arg Ser Ile Pro Phe Asp Gly Tyr Gln Leu Pro  
 565 570 575

Ala Asp Ala Ala Val Trp Met Arg Ile  
 580 585

<210> SEQ ID NO 31  
 <211> LENGTH: 1806  
 <212> TYPE: DNA  
 <213> ORGANISM: Bifidobacterium thermophilum

<400> SEQUENCE: 31

atggcagaac gcaagtcccc ccagtcocgc caagaatcca cggcatcggg tcgocgocga 60  
 gcggcctggt ggcacccaagc ggtggtctat caggtctatc cgcggttcgtt caaggacacc 120  
 accggttcgc gcctcggcga catcgcgggt gttaccagcc gcatcggcta cctcaagcaa 180  
 ctgggtgttg acgcgatttg gctcagcccc ttctatccga gccaaactcgc cgatggcggg 240  
 tacgatgtcg atgattaccg caacgtocgc ccgaaactcg gtacgatgga cgatttcgac 300  
 aaactcgcga aaaccgcgca cgaggccggt atcaagattg tcgtcgatat cgttcccaac 360  
 cattcttcca acctgcaccc ctggttcaag gccgcgcttg cagctggtcc gggctcgcgc 420  
 gaacgtgacc gctacatcct ccgtgacggc cgcggtgagc atggcgaact gccgcccacc 480  
 gactgggtgt ccacattcgg cggccccgcg tggacgcgcg tgctgacgg ccagtggtat 540  
 ctgcacctgt tcaccgtgga gcagcccgcac tggaaactgga agaaccggga tgtgcaggcg 600  
 gacttcatca agaccctgcg tttttggctt gatcacggcg ctgacggctt ccgtgtcgat 660  
 gttgcgcaag gcctgtgcaa ggacctgcac cgcgacaatc tcgaccagtg gagcgtcacc 720  
 ccgccaagcc tgcccgcgca cggcagccat ccgctgtacg accgcgacga cgtgcatcag 780  
 atttatcgcg agtggcgcaa ggtgttcaac gaatatgatc cgcggcatt cgcctgggcc 840  
 gaggcgtggg ttaatcccgc gcggcagtat ctgtatgcgt ccgacgatga gctcggctcag 900  
 gtgttcaact tcgagttcgc gaagaagaac tgggtgcgtg acgacatgca tcaggcgatc 960  
 gaggaaggtc tcgaggcggc gcgtcgttct ggctctaccg ccacgtgggt gatgagtaat 1020  
 cacgatgttc cacgtcagc cagccgttac gcaactgccg aagtgccgag cacgcggcat 1080  
 catcagttgg cgcagactg gctgctgcgt gacggcacca gctatcacga ggatcgcgaa 1140  
 gccggtacgc gccgcgcccg gcccgcgatt ttgatggagc ttgcggttgc cggttcggcg 1200  
 tacctgtacc agggcgaaga gcttggctcg ttcgaggtcg ctgatattcc gtggaacaag 1260  
 ctcgaggatc cgaccgcgcg caatagcgaa cgtgcggcca aggacaaggg gcgocgacggg 1320  
 tgccgcgtcc ccctgcgctg ggtcgcgcgc gacggggctc aggggtcgtt cggcttctcg 1380  
 cctcgtgtga aatccgtggg cgtcggcgtt tccgcgcatc aggcggggca gccgtcggag 1440  
 cctgcacacc tgccgcaacc cgcattggtc gctgatttcg ccgocgaccc tgagagcgcg 1500  
 cagccggagt cgatgtttaa cctgtaccgc agggcgttgg cgttgcccca tgagctgatg 1560  
 ccggccgaca caacgctgac ttggctggat gaagaccgcc cgtctgatgc gccggatggc 1620  
 gctgacggtc agcacggcgg cgtgattgct taccgcccgt ccaacggctg ggcgagtggt 1680  
 accaatttcg gtgcggaacc tgcgcattg ccggcggcg aggtgctgct cacctccggc 1740

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 gagctgtgct cgcagggccg gctgcccga gataccaccg tttggctgcg gttgaaccag 1800

gactga 1806

&lt;210&gt; SEQ ID NO 32

&lt;211&gt; LENGTH: 601

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Bifidobacterium thermophilum

&lt;400&gt; SEQUENCE: 32

Met Ala Glu Arg Lys Ser Pro Gln Ser Ala Gln Glu Ser Thr Ala Ser  
1 5 10 15Asp Arg Ala Ala Ala Trp Trp His Gln Ala Val Val Tyr Gln Val  
20 25 30Tyr Pro Arg Ser Phe Lys Asp Thr Thr Gly Ser Gly Leu Gly Asp Ile  
35 40 45Ala Gly Val Thr Ser Arg Ile Gly Tyr Leu Lys Gln Leu Gly Val Asp  
50 55 60Ala Ile Trp Leu Ser Pro Phe Tyr Pro Ser Gln Leu Ala Asp Gly Gly  
65 70 75 80Tyr Asp Val Asp Asp Tyr Arg Asn Val Asp Pro Lys Leu Gly Thr Met  
85 90 95Asp Asp Phe Asp Lys Leu Ala Lys Thr Ala His Glu Ala Gly Ile Lys  
100 105 110Ile Val Val Asp Ile Val Pro Asn His Ser Ser Asn Leu His Pro Trp  
115 120 125Phe Lys Ala Ala Leu Ala Ala Gly Pro Gly Ser Pro Glu Arg Asp Arg  
130 135 140Tyr Ile Phe Arg Asp Gly Arg Gly Glu His Gly Glu Leu Pro Pro Thr  
145 150 155 160Asp Trp Val Ser His Phe Gly Gly Pro Ala Trp Thr Arg Val Pro Asp  
165 170 175Gly Gln Trp Tyr Leu His Leu Phe Thr Val Glu Gln Pro Asp Trp Asn  
180 185 190Trp Lys Asn Pro Asp Val Gln Ala Asp Phe Ile Lys Thr Leu Arg Phe  
195 200 205Trp Leu Asp His Gly Ala Asp Gly Phe Arg Val Asp Val Ala His Gly  
210 215 220Leu Cys Lys Asp Leu Asp Arg Asp Asn Leu Asp Gln Trp Ser Val Thr  
225 230 235 240Pro Pro Ser Leu Pro Ala Asp Gly Ser His Pro Leu Tyr Asp Arg Asp  
245 250 255Asp Val His Gln Ile Tyr Arg Glu Trp Arg Lys Val Phe Asn Glu Tyr  
260 265 270Asp Pro Pro Ala Phe Ala Val Ala Glu Ala Trp Val Asn Pro Ala Arg  
275 280 285Gln Tyr Leu Tyr Ala Ser Asp Asp Glu Leu Gly Gln Val Phe Asn Phe  
290 295 300Glu Phe Ala Lys Lys Asn Trp Val Arg Asp Asp Met His Gln Ala Ile  
305 310 315 320Glu Glu Gly Leu Glu Ala Ala Arg Arg Ser Gly Ser Thr Ala Thr Trp  
325 330 335Val Met Ser Asn His Asp Val Pro Arg His Ala Ser Arg Tyr Ala Leu  
340 345 350

Pro Gln Val Pro Ser Thr Arg His His Gln Leu Ala His Asp Trp Leu

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355					360					365					
Leu	Arg	Asp	Gly	Thr	Ser	Tyr	His	Glu	Asp	Arg	Glu	Ala	Gly	Thr	Arg
370						375					380				
Arg	Ala	Arg	Ala	Ala	Ile	Leu	Met	Glu	Leu	Ala	Leu	Pro	Gly	Ser	Ala
385					390					395					400
Tyr	Leu	Tyr	Gln	Gly	Glu	Glu	Leu	Gly	Leu	Phe	Glu	Val	Ala	Asp	Ile
			405						410					415	
Pro	Trp	Asn	Lys	Leu	Glu	Asp	Pro	Thr	Ala	Arg	Asn	Ser	Glu	Arg	Ala
			420					425					430		
Ala	Lys	Asp	Lys	Gly	Arg	Asp	Gly	Cys	Arg	Val	Pro	Leu	Pro	Trp	Val
		435					440					445			
Ala	Ala	Asp	Gly	Val	Glu	Gly	Ser	Phe	Gly	Phe	Ser	Pro	Arg	Val	Lys
	450					455					460				
Ser	Val	Gly	Ala	Gly	Val	Ser	Ala	Asp	Gln	Ala	Gly	Gln	Pro	Ser	Glu
465						470					475				480
Pro	Ala	His	Leu	Pro	Gln	Pro	Ala	Trp	Phe	Ala	Asp	Phe	Ala	Ala	Asp
			485					490						495	
Arg	Glu	Ser	Ala	Gln	Pro	Glu	Ser	Met	Leu	Asn	Leu	Tyr	Arg	Arg	Ala
			500					505					510		
Leu	Ala	Leu	Arg	His	Glu	Leu	Met	Pro	Ala	Asp	Thr	Thr	Leu	Thr	Trp
		515					520						525		
Leu	Asp	Glu	Asp	Arg	Pro	Ser	Asp	Ala	Pro	Asp	Gly	Ala	Asp	Gly	Gln
	530					535					540				
His	Gly	Gly	Val	Ile	Ala	Tyr	Arg	Arg	Ser	Asn	Gly	Trp	Ala	Ser	Val
545					550					555					560
Thr	Asn	Phe	Gly	Ala	Glu	Pro	Val	Ala	Leu	Pro	Ala	Gly	Glu	Val	Leu
			565					570						575	
Leu	Thr	Ser	Gly	Glu	Leu	Cys	Ser	Asp	Gly	Arg	Leu	Pro	Gln	Asp	Thr
			580					585					590		
Thr	Val	Trp	Leu	Arg	Leu	Asn	Gln	Asp							
		595				600									

<210> SEQ ID NO 33  
 <211> LENGTH: 1803  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: BthGlu1\_codon-optimized

<400> SEQUENCE: 33

```

gtggctgaaa gaaaatcacc gcaaagcgca caggaaagca cggcctcaga cagagcagcc    60
gcagcctggt ggcacatcaggc cgtgtgtttat caagttttat ctagatcatt caaggataca    120
acgggctcag gactgggcca tattgcagga gttacaagca gaatcggcta ccttaagcaa    180
cttggagtcg atgctatttg gctgtcaccg ttttaccctg cacaactggc cgatggcggc    240
tacgatgttg atgactacag aaacctggat cctaaactgg gcacaatgga tgattttgat    300
aagctggcaa aaacagcaca tgaggcggga atcaagattg ttgttgatat cgttccgaat    360
catagctcaa atctgcaccc gtggtttaaa gcagcactgg cagccggacc gggcagcccc    420
gagagagata gatacatttt cagagacggc agaggcgaac atggcgaact gcctcctaca    480
gactggggtt cacatttttg aggaccggct tggacaagag ttcctgatgg ccagtggtag    540
ctgcatcttt ttacgggtga acagcctgat tgggaattgga aaaaccctga cgtccaggcg    600
gacttcatta aaacacttag attctggctg gaccatggag cggatggctt tagagttgac    660
    
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gttgcacatg gcctttgtaa ggacctgac agagacaacc ttgatcagtg gtcagttacg 720
ccgccttcac tgccctgctga cggatcacac ccgctttatg atagagacga cgttcatcag 780
atztatagag aatggagaaa agtttttaac gaatacgcac ctectgcggt tgcaaggcca 840
gaagcgtggg ttaatccggc tagacaatat ctttatgcaa gcgacgcaga gctgggacaa 900
gttttcaact ttgaatttgc caaaaagaat tgggtcagag atgatatgca tcaagcgatt 960
gaagaaggcc tggaagctgc tagaagaagc ggcagcacag caacatgggt tatgtcaaac 1020
catgatgtgc cgagacacgc gtcaagatac gcacttcctc aagttccgag cacaagacat 1080
caccaacttg ctcatgactg gcttctgaga gacggaacgt cataccacga ggatagagag 1140
gctggaacaa gaagagccag agccgcaatt ctgatggagc tggccctgcc gggatcagca 1200
tatctgtatc agggcgaaga actgggactg tttgaggttg cagacattcc gtggaataag 1260
ctggaagatc cgacagcaag aaattcagaa agagcggcga aagacaaagg aagagatgga 1320
tgtagagtgc cgctgccttg ggtcgcctgc gacggagttg agggctcatt tggattctca 1380
cctagagtta agagcgtcgg agcgggagtt tcagcagacc aggccggaca accgagcgaa 1440
cctgcacatc tgcctcagcc ggcattgggtc gccgatttcg cagccgacag agaactcagca 1500
caaccggagt caatgcttaa cctttacaga agagcgttg ctctgagaca tgaacttatg 1560
cctgccgata cgacactgac atggcttgac gaagatagac cttcagacgc accggacgga 1620
gcagacggac agcatggagg cgttattgca tatagaagat caaacggctg ggcaagcgtt 1680
acaaatttcg gagctgaacc tgtcgcgctt cctgctggcg aggttcttct tacgagcgga 1740
gaactgtgta gcatggcag acttccgcag gacacaacag tgtggcttag actgaatcag 1800
gat 1803

```

&lt;210&gt; SEQ ID NO 34

&lt;211&gt; LENGTH: 662

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Bifidobacterium breve

&lt;400&gt; SEQUENCE: 34

```

Met Tyr Phe His Ile Asn His Leu His Asp Thr Val Val Ile Asn Val
1           5           10          15
Ile Ser Lys His Gly Phe Thr Val Ala Val Arg Val Leu Leu Asn Pro
20          25          30
Ile Thr Thr Asn Pro Gln Gln Ser Gly Ala Thr His His Val Ser His
35          40          45
Thr Ile Thr His Ala Gln Lys Gly Ile Gly Met Thr Ala Asn Asn Leu
50          55          60
Asn Asp Asp Trp Trp Lys Gln Ala Val Val Tyr Gln Ile Tyr Pro Arg
65          70          75          80
Ser Phe Lys Asp Val Asn Gly Asp Gly Ile Gly Asp Ile Ala Gly Val
85          90          95
Thr Glu Lys Met Asp Tyr Leu Lys Asn Leu Gly Val Asp Ala Ile Trp
100         105         110
Leu Ser Pro Phe Tyr Pro Ser Asp Leu Ala Asp Gly Gly Tyr Asp Val
115         120         125
Ile Asp Tyr Arg Asn Val Asp Pro Arg Leu Gly Thr Met Asp Asp Phe
130         135         140
Asp Ala Met Ala Lys Ala Ala His Glu Ala Gly Ile Lys Val Ile Val
145         150         155         160
Asp Ile Val Pro Asn His Thr Ala Asp Lys His Val Phe Phe Lys Glu

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165				170				175							
Ala	Leu	Ala	Ala	Glu	Pro	Gly	Ser	Pro	Ala	Arg	Asp	Arg	Tyr	Ile	Phe
		180						185					190		
Arg	Asp	Gly	Arg	Gly	Glu	His	Gly	Glu	Leu	Pro	Pro	Asn	Asp	Trp	Gln
		195					200					205			
Ser	Phe	Phe	Gly	Gly	Pro	Ala	Trp	Ala	Arg	Val	Ala	Asp	Gly	Gln	Trp
	210					215					220				
Tyr	Leu	His	Leu	Phe	Asp	Lys	Ala	Gln	Pro	Asp	Val	Asn	Trp	Lys	Asn
	225				230						235				240
Pro	Asp	Ile	His	Glu	Glu	Phe	Lys	Lys	Thr	Leu	Arg	Phe	Trp	Ser	Asp
			245						250					255	
His	Gly	Thr	Asp	Gly	Phe	Arg	Ile	Asp	Val	Ala	His	Gly	Leu	Ala	Lys
			260					265					270		
Asp	Leu	Glu	Ser	Lys	Pro	Leu	Glu	Glu	Leu	Gly	Arg	Glu	Tyr	Ser	Val
		275					280					285			
Val	Gly	Val	Leu	Asn	His	Asp	Phe	Ser	His	Pro	Leu	Phe	Asp	Arg	Arg
	290					295					300				
Glu	Val	His	Asp	Ile	Tyr	Arg	Glu	Trp	Arg	Lys	Val	Phe	Asn	Glu	Tyr
	305				310					315					320
Asp	Pro	Pro	Arg	Phe	Ala	Val	Ala	Glu	Ala	Trp	Val	Val	Pro	Glu	His
			325						330					335	
Gln	His	Leu	Tyr	Ala	Ser	Met	Asp	Glu	Leu	Gly	Gln	Ser	Phe	Asn	Phe
		340						345					350		
Asp	Phe	Ala	Gln	Ala	Ser	Trp	Tyr	Ala	Asp	Glu	Phe	Arg	Ala	Ala	Ile
		355					360					365			
Ala	Ala	Gly	Leu	Lys	Ala	Ala	Ala	Glu	Thr	Gly	Gly	Ser	Thr	Thr	Thr
	370					375					380				
Trp	Val	Met	Asn	Asn	His	Asp	Val	Pro	Arg	Ser	Pro	Ser	Arg	Tyr	Gly
	385				390					395					400
Leu	Pro	Gln	Val	Lys	Gly	Ala	Pro	Tyr	His	Gln	Leu	Pro	His	Asp	Trp
			405						410					415	
Leu	Leu	Arg	Asn	Gly	Thr	Thr	Tyr	Pro	Glu	Asp	Arg	Glu	Leu	Gly	Thr
		420						425					430		
Arg	Arg	Ala	Arg	Ala	Ala	Ala	Ala	Leu	Met	Glu	Leu	Gly	Leu	Pro	Gly
		435						440				445			
Ala	Tyr	Ile	Tyr	Gln	Gly	Glu	Glu	Leu	Gly	Leu	Phe	Glu	Val	Ala	Asp
	450					455					460				
Ile	Pro	Trp	Asp	Arg	Leu	Glu	Asp	Pro	Thr	Ala	Phe	His	Thr	Ala	Gln
	465				470					475					480
Ala	Thr	Met	Asp	Lys	Gly	Arg	Asp	Gly	Cys	Arg	Val	Pro	Ile	Pro	Trp
			485						490					495	
Thr	Ala	Ala	Asn	Glu	Pro	Thr	Leu	Ala	Asp	Phe	Ser	Arg	Pro	Ile	Pro
			500					505					510		
Ala	Asp	Asp	Gly	Thr	Gly	Glu	Asn	His	Val	Pro	Leu	Cys	Ala	Ala	Gly
		515					520					525			
Gln	Phe	Gly	Thr	Gly	Ala	Ser	Phe	Gly	Phe	Ser	Pro	Ala	Thr	Arg	Ala
	530					535					540				
Glu	Gly	Val	Thr	Pro	Ala	Ala	Asp	Pro	His	Leu	Pro	Gln	Pro	Leu	Trp
	545				550					555				560	
Phe	Lys	Asp	Tyr	Ala	Val	Asp	Val	Glu	Gln	Ala	Asp	Pro	Asp	Ser	Met
			565						570					575	
Leu	Ala	Leu	Tyr	His	Ala	Ala	Leu	Ala	Ile	Arg	Gln	Glu	Ser	Leu	Thr
			580					585					590		

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Ala Thr Arg Asp Thr Thr Ala Glu Gln Val Asp Met Gly Pro Asp Val  
 595 600 605

Val Ala Tyr Thr Arg Ala Ala Val Gly Gly Arg Thr Phe Thr Ser Ile  
 610 615 620

Thr Asn Phe Gly Thr Glu Pro Val Glu Leu Pro Gly Gly Ser Val Val  
 625 630 635 640

Leu Thr Ser Gly Pro Leu Thr Pro Asp Gly Gln Leu Pro Thr Asp Thr  
 645 650 655

Ser Ala Trp Val Ile Lys  
 660

<210> SEQ ID NO 35  
 <211> LENGTH: 1812  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: BbrGlu2\_codon-optimized

<400> SEQUENCE: 35

gtgacagcaa ataaccttaa tgacgactgg tggaaacaag cagtgggtta ccaaatctat 60  
 cctagatcat tcaaggatgt taatggcgat ggaattggcg atattgcagg cgttacggaa 120  
 aaaatggatt acctgaaaaa tctgggagtt gacgctatct ggcttagccc ttttaaccg 180  
 agcgatctgg ccgacggagg ctatgatgtg attgactata gaaatgtga tccgagactg 240  
 ggaacgatgg atgatttoga tgcgatggct aaggcggcac atgaagcagg cattaagtt 300  
 attgttgata ttgtcccga tcatacagct gacaaacatg tctttttcaa agaagcactt 360  
 gcagcagaac cgggctcacc tgccagagac agatacatct ttagagatgg aagaggagaa 420  
 cacggcgaac ttccgcctaa tgactggcaa tcattctttg gcggaaccgc atgggctaga 480  
 gtcgcagatg gccaatggta ccttcatctt ttcgacaagg cgcaacctga tgtcaattgg 540  
 aaaaaccctg acattcaca agaattcaag aaaacgctga gatthtggtc agatcatggc 600  
 acggatggct tcagaatoga tgtggcacac ggacttgcaa aagatctgga aagcaaaccg 660  
 ctggaggaac ttggcagaga atatagcgtg gttggcgttc tgaacctga cttttcacat 720  
 ccgctgttcg atagaagaga agttcatgat atctacagag agtggagaaa agtttttaac 780  
 gaatatgacc cgccgagatt tgcggttga gaagcatggg tgggtgcctga acaccaacac 840  
 ctgtatgcat caatggacga gctgggacaa tcattcaact ttgattttgc acaggcatca 900  
 tggatgccc atgagtttag agctgccatt gccgcaggac ttaaggccgc agcggaaaca 960  
 ggaggctcaa cgacaacgtg ggtgatgaat aatcacgacg ttccctagaag cccgtcaaga 1020  
 tatggcctgc cgcaagttaa aggagcacct taccaccagc ttccgcacga ttggcttctg 1080  
 agaaatggaa caacatatcc ggaagataga gagcttgca cgagaagagc aagagcagcg 1140  
 gcactgatgg aactgggctc tctggcgca gcatacattt atcagggcga agaactggga 1200  
 ctttttgaag tggcggatat tccgtgggat agacttgagg acccgacagc attccacaca 1260  
 gcacaagcga caatggataa aggaagagac ggatgcagag tgcctattcc ttggacagct 1320  
 gcaaatgaac ctacactggc agacttcagc agaccgattc cggcagacga cggaacgggc 1380  
 gaaaaccatg tcccgctgtg cgctgcagga caattcgca caggagcgtc attcggttt 1440  
 agcccggcta caagagcga aggagttaca ccggtgcag atccgcatct gcctcaacct 1500  
 ctttggttta aagattatgc agttgatgtc gagcaggccg acccgattc aatgctggca 1560  
 ctgtatcatg cagcactggc gattagacaa gaatcactta cagcaacaag agacacgacg 1620

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gcggaacaag tggatatggg ccoctgacgtt gttgcatata cgagagcggc cgtgggcggc 1680
agaacattta catcaattac aaattttgga acggagcctg ttgaactgcc gggaggatca 1740
gttgttctga caagcggacc tctgacaccg gacggccaac tgcctacgga cacatcagct 1800
tgggtcatca aa 1812

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<210> SEQ ID NO 36
<211> LENGTH: 604
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: BbrGlu2 protein_encoded by optimized sequence

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<400> SEQUENCE: 36

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```

Met Thr Ala Asn Asn Leu Asn Asp Asp Trp Trp Lys Gln Ala Val Val
1           5           10          15
Tyr Gln Ile Tyr Pro Arg Ser Phe Lys Asp Val Asn Gly Asp Gly Ile
          20           25           30
Gly Asp Ile Ala Gly Val Thr Glu Lys Met Asp Tyr Leu Lys Asn Leu
          35           40           45
Gly Val Asp Ala Ile Trp Leu Ser Pro Phe Tyr Pro Ser Asp Leu Ala
          50           55           60
Asp Gly Gly Tyr Asp Val Ile Asp Tyr Arg Asn Val Asp Pro Arg Leu
65           70           75           80
Gly Thr Met Asp Asp Phe Asp Ala Met Ala Lys Ala Ala His Glu Ala
          85           90           95
Gly Ile Lys Val Ile Val Asp Ile Val Pro Asn His Thr Ala Asp Lys
          100          105          110
His Val Phe Phe Lys Glu Ala Leu Ala Ala Glu Pro Gly Ser Pro Ala
          115          120          125
Arg Asp Arg Tyr Ile Phe Arg Asp Gly Arg Gly Glu His Gly Glu Leu
          130          135          140
Pro Pro Asn Asp Trp Gln Ser Phe Phe Gly Gly Pro Ala Trp Ala Arg
          145          150          155          160
Val Ala Asp Gly Gln Trp Tyr Leu His Leu Phe Asp Lys Ala Gln Pro
          165          170          175
Asp Val Asn Trp Lys Asn Pro Asp Ile His Glu Glu Phe Lys Lys Thr
          180          185          190
Leu Arg Phe Trp Ser Asp His Gly Thr Asp Gly Phe Arg Ile Asp Val
          195          200          205
Ala His Gly Leu Ala Lys Asp Leu Glu Ser Lys Pro Leu Glu Glu Leu
          210          215          220
Gly Arg Glu Tyr Ser Val Val Gly Val Leu Asn His Asp Phe Ser His
          225          230          235          240
Pro Leu Phe Asp Arg Arg Glu Val His Asp Ile Tyr Arg Glu Trp Arg
          245          250          255
Lys Val Phe Asn Glu Tyr Asp Pro Pro Arg Phe Ala Val Ala Glu Ala
          260          265          270
Trp Val Val Pro Glu His Gln His Leu Tyr Ala Ser Met Asp Glu Leu
          275          280          285
Gly Gln Ser Phe Asn Phe Asp Phe Ala Gln Ala Ser Trp Tyr Ala Asp
          290          295          300
Glu Phe Arg Ala Ala Ile Ala Ala Gly Leu Lys Ala Ala Ala Glu Thr
          305          310          315          320

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Gly Gly Ser Thr Thr Thr Trp Val Met Asn Asn His Asp Val Pro Arg  
 325 330 335

Ser Pro Ser Arg Tyr Gly Leu Pro Gln Val Lys Gly Ala Pro Tyr His  
 340 345 350

Gln Leu Pro His Asp Trp Leu Leu Arg Asn Gly Thr Thr Tyr Pro Glu  
 355 360 365

Asp Arg Glu Leu Gly Thr Arg Arg Ala Arg Ala Ala Ala Leu Met Glu  
 370 375 380

Leu Gly Leu Pro Gly Ala Ala Tyr Ile Tyr Gln Gly Glu Glu Leu Gly  
 385 390 395 400

Leu Phe Glu Val Ala Asp Ile Pro Trp Asp Arg Leu Glu Asp Pro Thr  
 405 410 415

Ala Phe His Thr Ala Gln Ala Thr Met Asp Lys Gly Arg Asp Gly Cys  
 420 425 430

Arg Val Pro Ile Pro Trp Thr Ala Ala Asn Glu Pro Thr Leu Ala Asp  
 435 440 445

Phe Ser Arg Pro Ile Pro Ala Asp Asp Gly Thr Gly Glu Asn His Val  
 450 455 460

Pro Leu Cys Ala Ala Gly Gln Phe Gly Thr Gly Ala Ser Phe Gly Phe  
 465 470 475 480

Ser Pro Ala Thr Arg Ala Glu Gly Val Thr Pro Ala Ala Asp Pro His  
 485 490 495

Leu Pro Gln Pro Leu Trp Phe Lys Asp Tyr Ala Val Asp Val Glu Gln  
 500 505 510

Ala Asp Pro Asp Ser Met Leu Ala Leu Tyr His Ala Ala Leu Ala Ile  
 515 520 525

Arg Gln Glu Ser Leu Thr Ala Thr Arg Asp Thr Thr Ala Glu Gln Val  
 530 535 540

Asp Met Gly Pro Asp Val Val Ala Tyr Thr Arg Ala Ala Val Gly Gly  
 545 550 555 560

Arg Thr Phe Thr Ser Ile Thr Asn Phe Gly Thr Glu Pro Val Glu Leu  
 565 570 575

Pro Gly Gly Ser Val Val Leu Thr Ser Gly Pro Leu Thr Pro Asp Gly  
 580 585 590

Gln Leu Pro Thr Asp Thr Ser Ala Trp Val Ile Lys  
 595 600

<210> SEQ ID NO 37  
 <211> LENGTH: 1821  
 <212> TYPE: DNA  
 <213> ORGANISM: Bifidobacterium breve

<400> SEQUENCE: 37

atgactactt tcaaccgcgc aataattcct gacgccattc gcaccaacgg agccacgccc 60  
 aacccgtggg ggtcgaatgc ggtggtgtac cagatctacc cgcggtcgtt ccaggacacg 120  
 aacggcgatg gtctcggcga cctgaagggc atcacctccc gcctcgacta tcttgccgat 180  
 ctcggcgtgg atgtgctctg gctctccccg gtctacaggt ccccgcaaga cgacaacggc 240  
 tacgacatct ccgactaccg ggacatcgac ccgctgttcg gcacgcttga cgacatggac 300  
 gagctgctgg ccgaagcgca caagcgcggc ctcaagatcg tgatggacct ggtgggtcaac 360  
 cacacctcgg acgagcagcg gtggttcgag gcgtcgaagg acaaggacga cccgacgccc 420  
 gactgggtact ggtggcgctc cgcgccccc gccacagagc cgggcacgcc cggcgccgag 480  
 ccgaaccagt ggggctccta ctcggcgggc tccgcatggg agtacagccc ggagcggcgc 540

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gagtactacc tgcaccagtt ctgcaagaag cagcctgac tcaactggga gaaccgggc 600
gtgcccgtg cagtgtacga catgatgaat tgggtgctcg atcgggcat cgacggcttc 660
cgtatggacg tcataccoct tatctccaag cgtacggatg caaacggcag gctccccggc 720
gagtacgggt ccgagctoca tgacctgccg gtgggggagg agggctactc cagccccaat 780
ccgttctgtg ccgacggctc gcgtcaggac gagttcctcg ccgagatgcg ccgagagggtg 840
ttcgacgggc gtgacggctt cctcaccgtc ggcgaggccc ccgggatcac cgccgaacgc 900
aacgagcaca tcaccaatcc ggccaatggg gagctggata tgctgttctt gttcgaacat 960
gtcgatattg attgtgatgg cgtcaagtgg aagcctctgc cgctcgattt gccgggatc 1020
aagcggatca tggccggata tcagactgct gtggagaacg tgggctgggc aagcttggtc 1080
actggtaacc acgatcagcc acgtgtggtc tctcgttggg gcgatgactc ctcggaggaa 1140
tccccgctgc gctcggccaa agcgttggc ctgatgttgc acatgcatcg cggcaccccg 1200
tacgtatata agggtgagga gctgggcatg accaatgctc acttcaccag cctcgatcag 1260
taccgcgacc ttgaatctct caatgcctat cgtcagaggg tcgaggaagc caaggtacaa 1320
tcgccggaat cgatgatggc gggtatcgcc gcgcgggtc gcgacaattc gcgtacocca 1380
atgcaatggg atggttctgc ctatgcccgt ttcaccgcac cggatgcagc gacggagccc 1440
tggatttccg tcaaccgaa tcatgtgaa atcaatgcgc ccggcgaatt cgacgatcct 1500
gactcgggtg atgccttcta caagaagtc atgccttgc gccacaacag ttcgattgtg 1560
gcggctggcg agtggcggtt gattgatgcg gatgacgcgc atgtatatgc gttcaccgcg 1620
acgcttgga acgagcgatt gctggtgtg gttaacctgt ccggccgaac cgtcgacttg 1680
ccgcgtgaat ccaccgagct gattgcccgc ggcgtcactg agccagatat cattctctcc 1740
acgtacgacg cccctcacac tgtggtctcc ctcgccaacc gtgagcttga cccgtgggag 1800
gctgctgccc tccagctgta a 1821

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<210> SEQ ID NO 38
<211> LENGTH: 419
<212> TYPE: DNA
<213> ORGANISM: Bifidobacterium breve
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: n is a, c, g, or t
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: n is a, c, g, or t
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: n is a, c, g, or t
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (19)..(19)
<223> OTHER INFORMATION: n is a, c, g, or t
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (29)..(29)
<223> OTHER INFORMATION: n is a, c, g, or t
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (56)..(56)
<223> OTHER INFORMATION: n is a, c, g, or t
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (84)..(84)
<223> OTHER INFORMATION: n is a, c, g, or t
<220> FEATURE:

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<221> NAME/KEY: misc\_feature  
<222> LOCATION: (115)..(115)  
<223> OTHER INFORMATION: n is a, c, g, or t  
<220> FEATURE:  
<221> NAME/KEY: misc\_feature  
<222> LOCATION: (136)..(136)  
<223> OTHER INFORMATION: n is a, c, g, or t  
<220> FEATURE:  
<221> NAME/KEY: misc\_feature  
<222> LOCATION: (138)..(138)  
<223> OTHER INFORMATION: n is a, c, g, or t  
<220> FEATURE:  
<221> NAME/KEY: misc\_feature  
<222> LOCATION: (149)..(149)  
<223> OTHER INFORMATION: n is a, c, g, or t  
<220> FEATURE:  
<221> NAME/KEY: misc\_feature  
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<221> NAME/KEY: misc\_feature  
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<221> NAME/KEY: misc\_feature  
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<223> OTHER INFORMATION: n is a, c, g, or t  
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<221> NAME/KEY: misc\_feature  
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<223> OTHER INFORMATION: n is a, c, g, or t  
<220> FEATURE:  
<221> NAME/KEY: misc\_feature  
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<221> NAME/KEY: misc\_feature  
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<223> OTHER INFORMATION: n is a, c, g, or t  
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<221> NAME/KEY: misc\_feature  
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<220> FEATURE:  
<221> NAME/KEY: misc\_feature  
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&lt;400&gt; SEQUENCE: 38

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dyrddgtddm daahkrqkvm dvvnhtsdha waskdkddha dwywwrargh gtganwgsyg      120
gsawysrgyy hskkdnwnav rravydmmnw wdrgdgrmdv tskrt dangr gygshdvgyy      180
ssncadgrda mrrvdgrdgt vgagtarnht nangdmhvdd cdgvkwkdgk rmagytavnv      240
gwastgnhdr vvsrwgddss srvsakagm hmhrqtyvyg gmtnahtsdy rdsnayrrva      300
kvssmmagaa rgrdnrtmw dgsayagtad aatwsvnnha naagdddsvy aykkarhnss      360
vaagwradd ahvyatrtgn rvvnsgrtv drstaggvtd stydahtvvs anrdwaaav      419

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&lt;210&gt; SEQ ID NO 39

&lt;211&gt; LENGTH: 1818

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: BbrGlu5\_codon-optimized

&lt;400&gt; SEQUENCE: 39

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aatccgtggg ggtcaaaccg agtggctcat caaatctatc cgagatcatt tcaagacaca      120
aacggagacg gccttgccga tcttaaggga atcacatcaa gactggatta tctggctgac      180
cttgagttg atgttctgtg gctgagcccg gtttatagat cacctcaaga cgacaatggc      240
tatgacatca gcgactatag agacattgat cctctgtttg gcacactgga tgatattgac      300
gagctgcttg cagaagcaca taaaagagga cttaaaatcg ttatggacct ggttgtgaac      360
catacatcag atgaacacgc atggttgaa gcatcaaaag ataaagacga tccgcaacgct      420
gactggattt ggtggagacc tgctagaccg ggccatgaac cgggaacacc tggcgagag      480
ccgaaccaat ggggctcata ttttgccgga tcagcatggg agtatagccc ggaagaggc      540
gaatactatc ttcacagtt ctcaaaaaa caaccggatc tgaattggga aaatccggcg      600
gtcagaagag cgggtgacga tatgatgaac tgggtggctgg atagaggaat tgatggattt      660
agaatggatg ttattacact gatttcaaaa agaacagacg ccaatggaag acttccggga      720
gaatatggat cagaactgca cgacctcct gtggcggaag agggctattc atcacctaat      780
cgtttttgcg ccgacggccc gagacaagat gaattccttg ccgaaatgag aagagaagtt      840
tttgacggaa gagatggctt tctgacagtc ggccaagcac ctggaattac agcagaaaga      900
aacgaacaca ttacaaacc tgcaaacggc gaacttgata tgctgttctt gtttgaacat      960
gtggactttg attgcatggc cgttaaatgg aaaccgcttc cgctggatct tcttgcttt      1020
aaaagaatta tggcaggcta tcagacagca gttgaaaatg tcggatgggc atcactgtt      1080
acaggcaatc atgaccaacc gagagtgtc agcagatggg gcgatgactc atcagaggag      1140
agcagagtta gaagcgccaa agcactgggc cttatgctgc acatgcacag aggcacaccg      1200
tatgtttatc aaggcgagga acttggatg acaaatgctc attttacgct acttgaccag      1260
tacagagatc ttgagtcact taatgcttat agacaaagag ttgaagaagc caaggttcag      1320
tcacctgaaa gcatgatggc cggcattgca gctagaggca gagataattc aagaacgccg      1380
atgcaatggg atggaagcgc atacgcaggc tttacggcac ctgacgcagc tacggaaccg      1440
tggatttcag ttaatccgaa tcatgcagaa attaacgcag caggagaatt tgatgacccg      1500
gattcagctc atgcattota caaaaaactg attgcaactg acataaatag cagcattgtt      1560

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gcagcgggagc aatggagact tatcgatgca gacgatgcac acgtttatgc gtttacaaga 1620
acacttgcca acgagagact gcttgctgtg gttaatctga gcggcagaac agttgatctg 1680
ccgagagagt caacagagct tattgctggc ggcgtgacag aaccggacat tattctttca 1740
acatatgaag cccctcatac agtgggtttca ctggcaaata gagagctgga cccgtgggaa 1800
gctgcggcag tgcagctg 1818

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<210> SEQ ID NO 40
<211> LENGTH: 1570
<212> TYPE: PRT
<213> ORGANISM: Streptococcus sp C150

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<400> SEQUENCE: 40

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Thr Val Ala Gly Gly Thr Leu Leu Glu Asp Gln Gln Val Gln Ala Asp
20      25      30
Glu Gln Asn Ala Ala Asn Gln Ser Gly Asp Ser Ser Gln Asp Leu Leu
35      40      45
Arg Glu Ala Ser Ala Thr Thr Asn Asp Thr Ala Thr Thr Val Ala Pro
50      55      60
Thr Ile Ser Ala Asp Ala Asn Thr Ala Ser Val Asn Ile Pro Val Ala
65      70      75      80
Asp Ala Thr Asn Thr Thr Thr Ala Ala Thr Asp Arg Ala Ala Ala Pro
85      90      95
Thr Thr Thr Ala Ala Thr Val Asp Thr Asn Ser Gly Gln Ala Ala Pro
100     105     110
Ser Thr Asn Val Gln Ala Ala Ala Ala Asp Thr Ser Ala Thr Pro Thr
115     120     125
Asp Thr Asn Thr Asn Thr Asn Ala Ser Val Thr Ala Thr Asp Arg Ala
130     135     140
Val Ala Thr Asp Thr Ala Asn Thr Glu Ala Arg Thr Pro Ser Arg Arg
145     150     155     160
Ala Leu Ala Glu Thr Arg Glu Ala Asn Thr Asn Thr Ser Thr Gly Ile
165     170     175
Gln Trp Ile Asn Gly Lys Gln Tyr Tyr Val Asn Ser Asp Gly Ser Val
180     185     190
Arg Lys Asn Phe Val Phe Glu Gln Asp Gly Lys Ser Tyr Tyr Phe Asp
195     200     205
Ala Glu Thr Gly Ala Leu Ala Thr Lys Ser Gln Asp Glu Phe Ser Thr
210     215     220
Glu Pro Ile Lys Ala Ala Val Asp Phe Ser Ser Gly Asn Gln Leu Tyr
225     230     235     240
Lys Asn Asp Asn Lys Ser Leu Asp Gln Leu Asp Thr Phe Ile Thr Ala
245     250     255
Asp Ala Trp Tyr Arg Pro Lys Ser Ile Leu Lys Asp Gly Lys Thr Trp
260     265     270
Thr Ala Ser Thr Glu Ala Asp Lys Arg Pro Leu Leu Met Val Trp Trp
275     280     285
Pro Asp Lys Ser Thr Gln Val Asn Tyr Leu Asn Tyr Met Gln Asn Gln
290     295     300
Gly Leu Gly Ala Gly Ser Phe Ser Thr Asn Ser Ser Gln Glu Ser Leu
305     310     315     320
Asn Leu Ala Ala Lys Ala Val Gln Thr Lys Ile Glu Glu Arg Ile Ala

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Lys	Thr	Gln	Pro	Gly	Trp	Asn	Ser	Ser	Thr	Glu	Asn	Ser	Ser	Tyr	Asp
		355					360						365		
His	Leu	Gln	Gly	Gly	Gln	Leu	Leu	Phe	Asn	Asn	Ser	Lys	Gly	Asp	Thr
	370					375						380			
Gly	Asn	Arg	Thr	Ser	Tyr	Ala	Asn	Ser	Asp	Tyr	Arg	Leu	Leu	Asn	Arg
385					390					395					400
Thr	Pro	Thr	Asn	Gln	Ser	Gly	Thr	Arg	Lys	Tyr	Phe	Lys	Asp	Asn	Ser
				405					410						415
Ile	Gly	Gly	Leu	Glu	Phe	Leu	Leu	Ala	Asn	Asp	Ile	Asp	Asn	Ser	Asn
			420					425						430	
Pro	Ala	Val	Gln	Ala	Glu	Gln	Leu	Asn	Trp	Leu	His	Phe	Met	Met	Asn
		435						440					445		
Ile	Gly	Ser	Ile	Met	Ala	Asn	Asp	Pro	Thr	Ala	Asn	Phe	Asp	Gly	Leu
450						455						460			
Arg	Val	Asp	Ala	Leu	Asp	Asn	Val	Asp	Ala	Asp	Leu	Leu	Gln	Ile	Ala
465					470					475					480
Ser	Asp	Tyr	Phe	Lys	Ala	Val	Tyr	Gly	Val	Asp	Lys	Ser	Glu	Ala	Asn
				485					490						495
Ala	Ile	Lys	His	Leu	Ser	Tyr	Leu	Glu	Ala	Trp	Ser	Ala	Asn	Asp	Pro
			500					505						510	
Tyr	Tyr	Asn	Lys	Asp	Thr	Lys	Gly	Ala	Gln	Leu	Pro	Ile	Asp	Asn	Ala
		515					520						525		
Leu	Arg	Asn	Ala	Leu	Thr	Asn	Leu	Leu	Met	Arg	Asp	Lys	Asn	Thr	Arg
530						535					540				
Met	Gln	Leu	Gly	Asp	Met	Thr	Ala	Phe	Met	Asn	Ser	Ser	Leu	Asn	Pro
545					550					555					560
Arg	Gly	Ala	Asn	Asp	Lys	Asn	Gly	Glu	Arg	Met	Ala	Asn	Tyr	Ile	Phe
				565					570						575
Thr	Arg	Ala	His	Asp	Thr	Glu	Ala	Gln	Thr	Ile	Ile	Gln	Arg	Ile	Ile
			580					585						590	
Arg	Asp	Arg	Ile	Asn	Pro	Asn	Leu	Phe	Gly	Tyr	Asn	Phe	Thr	Arg	Asp
		595					600					605			
Glu	Ile	Lys	Lys	Ala	Phe	Glu	Ile	Tyr	Asn	Ala	Asp	Ile	Asn	Thr	Ala
610						615						620			
His	Lys	Thr	Tyr	Ala	Ser	Tyr	Asn	Leu	Pro	Ser	Val	Tyr	Ala	Leu	Met
625					630						635				640
Leu	Thr	Asn	Lys	Asp	Ser	Val	Thr	Arg	Val	Tyr	Tyr	Gly	Asp	Leu	Tyr
				645					650						655
Arg	Glu	Asp	Gly	His	Tyr	Met	Ala	Lys	Lys	Thr	Pro	Tyr	Phe	Asp	Ala
			660					665						670	
Ile	Asp	Thr	Leu	Leu	Arg	Ala	Arg	Ile	Lys	Tyr	Val	Ala	Gly	Gly	Gln
		675					680						685		
Asp	Met	Glu	Val	Lys	Lys	Val	Gly	Asn	Asp	Gly	Leu	Leu	Thr	Ser	Val
690						695					700				
Arg	Tyr	Gly	Lys	Gly	Ala	Asn	Asn	Ser	Thr	Asp	Trp	Gly	Thr	Thr	Glu
705					710					715					720
Thr	Arg	Thr	Gln	Gly	Met	Gly	Val	Ile	Leu	Thr	Asn	Asn	Tyr	Asp	Phe
				725					730						735
Arg	Leu	Gly	Ser	Asn	Glu	Thr	Val	Thr	Met	Asn	Met	Gly	Arg	Ala	His
			740					745							750

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Arg Asn Gln Leu Tyr Arg Pro Leu Leu Leu Thr Thr Lys Asp Gly Leu  
 755 760 765

Ala Thr Tyr Leu Asn Asp Ser Asp Val Pro Ser Asn Leu Leu Lys Arg  
 770 775 780

Thr Asp Trp Asn Gly Asn Leu Thr Phe Asn Ala Asn Asp Val Phe Gly  
 785 790 795 800

Val Glu Asn Val Gln Val Ser Gly Tyr Leu Gly Val Trp Val Pro Val  
 805 810 815

Gly Ala Lys Ala Asn Gln Asp Ala Arg Thr Gln Pro Ser Asn Arg Ala  
 820 825 830

Asn Ser Asp Gly Gln Val Tyr Lys Ser Ser Ala Ala Leu Asp Ser Gln  
 835 840 845

Val Met Tyr Glu Ala Phe Ser Asn Phe Gln Ala Phe Ala Asp Asp Gln  
 850 855 860

Pro Glu Leu Tyr Met Asn Arg Val Leu Ala Lys Asn Thr Asp Leu Leu  
 865 870 875 880

Lys Ala Trp Gly Val Thr Ser Val Gly Leu Pro Pro Gln Tyr Val Ser  
 885 890 895

Ser Lys Asp Gly Thr Phe Leu Asp Ser Thr Ile Asp Asn Gly Tyr Ala  
 900 905 910

Phe Asp Asp Arg Tyr Asp Met Ala Leu Ser Gln Asn Asn Lys Tyr Gly  
 915 920 925

Ser Leu Glu Asp Leu Leu Asn Val Leu Arg Ala Leu His Lys Asp Gly  
 930 935 940

Ile Gln Ala Ile Ala Asp Trp Val Pro Asp Gln Ile Tyr Asn Leu Pro  
 945 950 955 960

Gly Lys Glu Val Val Asn Ala Thr Arg Val Asn Gly Tyr Gly Tyr His  
 965 970 975

Gln Gln Gly Tyr Gln Ile Val Asp Gln Ala Tyr Val Ala Asn Thr Arg  
 980 985 990

Thr Asp Gly Thr Asp Tyr Gln Gly Arg Tyr Gly Gly Ala Phe Leu Asp  
 995 1000 1005

Glu Leu Lys Ala Lys Tyr Pro Ser Ile Phe Asn Arg Val Gln Ile  
 1010 1015 1020

Ser Asn Gly Lys Gln Leu Pro Thr Asn Glu Lys Ile Thr Lys Trp  
 1025 1030 1035

Ser Ala Lys Tyr Phe Asn Gly Thr Asn Ile Leu Gly Arg Gly Ile  
 1040 1045 1050

Asn Tyr Val Leu Arg Asp Asp Lys Thr Asn Gln Tyr Phe Asn Thr  
 1055 1060 1065

Ser Ala Asn Gly Gln Leu Leu Pro Thr Pro Leu Arg Asp Thr Gly  
 1070 1075 1080

Ala Ile Thr Ser Thr Gln Val Phe Gln Arg Arg Gly Gln Asp Val  
 1085 1090 1095

Tyr Phe Leu Arg Asp Asn Gln Val Ile Lys Asn Glu Phe Val Gln  
 1100 1105 1110

Asp Gly Asn Gly Asn Trp Tyr Tyr Phe Gly Ala Asp Gly Lys Met  
 1115 1120 1125

Thr Lys Gly Ala Gln Asn Ile Asn Ser Lys Asp Tyr Tyr Phe Phe  
 1130 1135 1140

Asp Asn Gly Val Gln Leu Arg Asn Ala Leu Arg Arg Ala Ser Asn  
 1145 1150 1155

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Gly	Tyr	Thr	Tyr	Tyr	Tyr	Gly	Leu	Asp	Gly	Ala	Met	Ile	Lys	Asn
1160						1165					1170			
Ala	Phe	Val	Asp	Phe	Asp	Asp	Lys	His	Gln	Gln	Val	Arg	Ala	Phe
1175						1180					1185			
Thr	Thr	Gln	Gly	Thr	Met	Val	Val	Gly	Asn	Leu	His	Trp	Ser	Gly
1190						1195					1200			
His	His	Phe	Tyr	Phe	Asp	Arg	Glu	Thr	Gly	Ile	Gln	Ala	Lys	Asp
1205						1210					1215			
Arg	Ile	Val	Arg	Thr	Asp	Asp	Gly	Lys	Leu	His	Tyr	Tyr	Val	Ala
1220						1225					1230			
Gln	Thr	Gly	Asp	Met	Gly	Arg	Asn	Val	Phe	Ala	Thr	Asp	Ser	Arg
1235						1240					1245			
Thr	Gly	Lys	Arg	Tyr	Tyr	Phe	Asp	Ala	Asp	Gly	Asn	Thr	Val	Thr
1250						1255					1260			
Gly	Ser	Arg	Val	Ile	Asp	Gly	Lys	Thr	Tyr	Tyr	Phe	Asn	Gln	Asp
1265						1270					1275			
Gly	Ser	Val	Gly	Thr	Ala	Tyr	Ser	Asn	Arg	Ala	Asp	Ser	Ile	Ile
1280						1285					1290			
Phe	Glu	Asn	Gly	Lys	Ala	Arg	Tyr	Ile	Thr	Pro	Ala	Gly	Glu	Ile
1295						1300					1305			
Gly	Arg	Ser	Ile	Phe	Val	Tyr	Asn	Pro	Ala	Thr	Lys	Ala	Trp	Asn
1310						1315					1320			
Tyr	Phe	Asp	Lys	Glu	Gly	Asn	Arg	Val	Thr	Gly	Arg	Gln	Tyr	Ile
1325						1330					1335			
Asp	Gly	Asn	Leu	Tyr	Tyr	Phe	Lys	Glu	Asp	Gly	Ser	Gln	Val	Lys
1340						1345					1350			
Gly	Ala	Ile	Val	Glu	Glu	Asn	Gly	Ile	Lys	Tyr	Tyr	Tyr	Glu	Pro
1355						1360					1365			
Gly	Ser	Gly	Ile	Leu	Ala	Ser	Gly	Arg	Tyr	Leu	Gln	Val	Gly	Asp
1370						1375					1380			
Asp	Gln	Trp	Ile	Tyr	Phe	Lys	His	Asp	Gly	Ser	Leu	Ala	Ile	Gly
1385						1390					1395			
Gln	Val	Arg	Ala	Asp	Gly	Gly	Tyr	Leu	Lys	Tyr	Phe	Asp	Lys	Asn
1400						1405					1410			
Gly	Ile	Gln	Val	Lys	Gly	Gln	Thr	Ile	Val	Glu	Asp	Gly	His	Thr
1415						1420					1425			
Tyr	Tyr	Tyr	Asp	Ala	Asp	Ser	Gly	Ala	Leu	Val	Thr	Ser	Ser	Phe
1430						1435					1440			
Ala	Glu	Ile	Ala	Pro	Asn	Gln	Trp	Ala	Tyr	Phe	Asn	Thr	Glu	Gly
1445						1450					1455			
Gln	Ala	Leu	Lys	Gly	Lys	Trp	Thr	Ile	Asn	Gly	Lys	Glu	Tyr	Tyr
1460						1465					1470			
Phe	Asp	Gln	Asn	Gly	Ile	Gln	Tyr	Lys	Gly	Lys	Ala	Val	Lys	Val
1475						1480					1485			
Gly	Ser	Arg	Tyr	Lys	Tyr	Tyr	Asp	Glu	Asn	Asp	Gly	Gln	Pro	Val
1490						1495					1500			
Thr	Asn	Arg	Phe	Ala	Gln	Ile	Glu	Pro	Asn	Val	Trp	Ala	Tyr	Phe
1505						1510					1515			
Gly	Ala	Asp	Gly	Tyr	Ala	Val	Thr	Gly	Glu	Gln	Val	Ile	Asn	Gly
1520						1525					1530			
Gln	His	Leu	Tyr	Phe	Asp	Gln	Ser	Gly	Arg	Gln	Val	Lys	Gly	Ala
1535						1540					1545			
Tyr	Val	Thr	Val	Asn	Gly	Gln	Arg	Arg	Tyr	Tyr	Asp	Ala	Asn	Thr

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gcacacgata ccgaggcgca gaccatcatt cagcgtatta tccgcgatcg tatcaatccg			1260
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ggcttctgta cgagcgtccg ctatggcaag ggtgcgaaca atagcaccga ctggggcacg			1620
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tataagtcgt ctgcggcatt ggacagccag gtcattgatg aggcgtttag caattttcag	2040
gcatttgcgg acgatcaacc ggaactgtac atgaaccgcg ttctggcgaa gaacaccgat	2100
ctgctgaaag cgtggggcgt tactagcgtt ggcttgccgc cacaatacgt tagcagcaaa	2160
gacggcacct tcctggatag cactattgat aacggctatg cgttcogatga tcgttaacgac	2220
atggcgctga gccagaacaa caaatacggg tctctggagg acttgctgaa cgttctgcgc	2280
gctctgcaca aagacgggat tcaggcgatt gcggactggg tcccgatca aatctacaat	2340
ttgccgggta aagaggttgt taatgcgacg cgtgttaacg gttacgggta ccatcagcag	2400
ggctaccaga ttgttgacca ggcgtacgtt gcaaaccacc gtacggatgg taccgattat	2460
cagggtcgtt acgggtgtgc ttttctggac gaactgaagg cgaagtaccc gagcattttc	2520
aatcgtgtcc agattagcaa cggtaaacag ctgccaacca atgagaaat cacgaaatgg	2580
tccgcgaaat acttcaatgg cacgaacatc ctgggccgtg gtattaacta tgtgctgcgc	2640
gacgacaaga ccaatcagta tttcaacacc agcgcaaacg gccaaactgt gccgacgcca	2700
ctgcgcgaca ccggtgccat caccagcagc caagttttcc agcgtcgtgg ccaagacgtc	2760
tattttctgc gtgataacca ggttatcaaa aacgagtttg tgcaagatgg taacggtaat	2820
tggtactact tcggtgccga cggtaaaatg acgaagggtg cacaaaacat caatagcaag	2880
gattactatt tcttcgataa tggcgtccag ctgcgtaatg cgctgcgtcg cgcgtccaat	2940
ggttacacct actattatgg cctggacggg gccatgatca agaacgcttt cgtcogatttt	3000
gatgataaag accaacaggt gcgtgcgttt actacgcagg gcacgatggg ggtcggtaat	3060
ttgcactgga gcggtcacca cttctatttt gaccgcgaaa cgggtatcca agccaaagac	3120
cgcatgtgac gtaccgatga tggcaagctg cactattatg tcgcacaaac cggcgatatg	3180
ggccgcaatg tgtttgcgac cgacagccgc acgggcaagc gctattactt tgatgcggac	3240
ggcaacaccc ttacgggctc ccgtgtcatc gacggcaaga cctactactt caaccaggac	3300
ggttcggtcg gtaccgcgta cagcaatcgt gcggatagca ttatctttga gaatggcaag	3360
gctcgtctata tcaactccgc tggcgagatt ggccgttcca tttttgtcta caaccggcg	3420
accaaagcgt ggaattactt cgacaaggaa ggtaaccgtg tcaccggtcg tcagtatatt	3480
gacggcaatc tgtactactt taaagaggac ggctcccaag tgaaaggtgc gattgttgaa	3540
gagaacggta tcaagtacta ctacgaaccg ggcagcggta tcctggcgag cggtcgttat	3600
ctgcaagtgc gtgacgacca atggatctac ttcaaacacg acggtagcct ggcgatcggg	3660
caggttcgtg cagacgggtg ttacttgaaa tactttgata agaatggcat ccaggtaacg	3720
ggccaaacca ttgtggagga tggtcatacc tattactacg atgcccactc cggtgctctg	3780
gtgacctcta gcttcgcgga gattgctccg aaccagtggt cctacttcaa taccgagggc	3840
caagccctga agggcaaatg gaccatcaat ggtaagagt actattttga tcagaacggc	3900
attcagtata aaggcaaggc agttaaggtc ggcagccgtt acaaatacta tgacgagaat	3960
gacggtaaac cggtcactaa ccgttttgcc cagattgagc cgaacgtctg ggcgtacttt	4020
ggtgccgatg gctacgcagt tactggcgaa caggtgatta atggccagca cctgtaactc	4080
gatcagtcgg gtcgtcaggt taaaggtgcg tacgtcaccg tgaatggta acgtcgttac	4140
tacgacgcaa acacgggtga atacattccg ggtcgttaa	4179

&lt;210&gt; SEQ ID NO 42

&lt;211&gt; LENGTH: 1392

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Streptococcus sp C150

-continued

&lt;400&gt; SEQUENCE: 42

Ile Asn Gly Lys Gln Tyr Tyr Val Asn Ser Asp Gly Ser Val Arg Lys  
 1 5 10 15  
 Asn Phe Val Phe Glu Gln Asp Gly Lys Ser Tyr Tyr Phe Asp Ala Glu  
 20 25 30  
 Thr Gly Ala Leu Ala Thr Lys Ser Gln Asp Glu Phe Ser Thr Glu Pro  
 35 40 45  
 Ile Lys Ala Ala Val Asp Phe Ser Ser Gly Asn Gln Leu Tyr Lys Asn  
 50 55 60  
 Asp Asn Lys Ser Leu Asp Gln Leu Asp Thr Phe Ile Thr Ala Asp Ala  
 65 70 75 80  
 Trp Tyr Arg Pro Lys Ser Ile Leu Lys Asp Gly Lys Thr Trp Thr Ala  
 85 90 95  
 Ser Thr Glu Ala Asp Lys Arg Pro Leu Leu Met Val Trp Trp Pro Asp  
 100 105 110  
 Lys Ser Thr Gln Val Asn Tyr Leu Asn Tyr Met Gln Asn Gln Gly Leu  
 115 120 125  
 Gly Ala Gly Ser Phe Ser Thr Asn Ser Ser Gln Glu Ser Leu Asn Leu  
 130 135 140  
 Ala Ala Lys Ala Val Gln Thr Lys Ile Glu Glu Arg Ile Ala Arg Glu  
 145 150 155 160  
 Gly Asn Thr Asn Trp Leu Arg Thr Ser Ile Asp Gln Phe Ile Lys Thr  
 165 170 175  
 Gln Pro Gly Trp Asn Ser Ser Thr Glu Asn Ser Ser Tyr Asp His Leu  
 180 185 190  
 Gln Gly Gly Gln Leu Leu Phe Asn Asn Ser Lys Gly Asp Thr Gly Asn  
 195 200 205  
 Arg Thr Ser Tyr Ala Asn Ser Asp Tyr Arg Leu Leu Asn Arg Thr Pro  
 210 215 220  
 Thr Asn Gln Ser Gly Thr Arg Lys Tyr Phe Lys Asp Asn Ser Ile Gly  
 225 230 235 240  
 Gly Leu Glu Phe Leu Leu Ala Asn Asp Ile Asp Asn Ser Asn Pro Ala  
 245 250 255  
 Val Gln Ala Glu Gln Leu Asn Trp Leu His Phe Met Met Asn Ile Gly  
 260 265 270  
 Ser Ile Met Ala Asn Asp Pro Thr Ala Asn Phe Asp Gly Leu Arg Val  
 275 280 285  
 Asp Ala Leu Asp Asn Val Asp Ala Asp Leu Leu Gln Ile Ala Ser Asp  
 290 295 300  
 Tyr Phe Lys Ala Val Tyr Gly Val Asp Lys Ser Glu Ala Asn Ala Ile  
 305 310 315 320  
 Lys His Leu Ser Tyr Leu Glu Ala Trp Ser Ala Asn Asp Pro Tyr Tyr  
 325 330 335  
 Asn Lys Asp Thr Lys Gly Ala Gln Leu Pro Ile Asp Asn Ala Leu Arg  
 340 345 350  
 Asn Ala Leu Thr Asn Leu Leu Met Arg Asp Lys Asn Thr Arg Met Gln  
 355 360 365  
 Leu Gly Asp Met Thr Ala Phe Met Asn Ser Ser Leu Asn Pro Arg Gly  
 370 375 380  
 Ala Asn Asp Lys Asn Gly Glu Arg Met Ala Asn Tyr Ile Phe Thr Arg  
 385 390 395 400  
 Ala His Asp Thr Glu Ala Gln Thr Ile Ile Gln Arg Ile Ile Arg Asp

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405				410				415							
Arg	Ile	Asn	Pro	Asn	Leu	Phe	Gly	Tyr	Asn	Phe	Thr	Arg	Asp	Glu	Ile
		420						425					430		
Lys	Lys	Ala	Phe	Glu	Ile	Tyr	Asn	Ala	Asp	Ile	Asn	Thr	Ala	His	Lys
		435					440					445			
Thr	Tyr	Ala	Ser	Tyr	Asn	Leu	Pro	Ser	Val	Tyr	Ala	Leu	Met	Leu	Thr
	450				455						460				
Asn	Lys	Asp	Ser	Val	Thr	Arg	Val	Tyr	Tyr	Gly	Asp	Leu	Tyr	Arg	Glu
465					470					475					480
Asp	Gly	His	Tyr	Met	Ala	Lys	Lys	Thr	Pro	Tyr	Phe	Asp	Ala	Ile	Asp
				485					490					495	
Thr	Leu	Leu	Arg	Ala	Arg	Ile	Lys	Tyr	Val	Ala	Gly	Gly	Gln	Asp	Met
			500					505					510		
Glu	Val	Lys	Lys	Val	Gly	Asn	Asp	Gly	Leu	Leu	Thr	Ser	Val	Arg	Tyr
		515					520						525		
Gly	Lys	Gly	Ala	Asn	Asn	Ser	Thr	Asp	Trp	Gly	Thr	Thr	Glu	Thr	Arg
	530					535					540				
Thr	Gln	Gly	Met	Gly	Val	Ile	Leu	Thr	Asn	Asn	Tyr	Asp	Phe	Arg	Leu
545					550					555					560
Gly	Ser	Asn	Glu	Thr	Val	Thr	Met	Asn	Met	Gly	Arg	Ala	His	Arg	Asn
			565						570					575	
Gln	Leu	Tyr	Arg	Pro	Leu	Leu	Leu	Thr	Thr	Lys	Asp	Gly	Leu	Ala	Thr
			580					585					590		
Tyr	Leu	Asn	Asp	Ser	Asp	Val	Pro	Ser	Asn	Leu	Leu	Lys	Arg	Thr	Asp
		595					600					605			
Trp	Asn	Gly	Asn	Leu	Thr	Phe	Asn	Ala	Asn	Asp	Val	Phe	Gly	Val	Glu
	610					615					620				
Asn	Val	Gln	Val	Ser	Gly	Tyr	Leu	Gly	Val	Trp	Val	Pro	Val	Gly	Ala
625					630					635					640
Lys	Ala	Asn	Gln	Asp	Ala	Arg	Thr	Gln	Pro	Ser	Asn	Arg	Ala	Asn	Ser
			645						650					655	
Asp	Gly	Gln	Val	Tyr	Lys	Ser	Ser	Ala	Ala	Leu	Asp	Ser	Gln	Val	Met
		660						665					670		
Tyr	Glu	Ala	Phe	Ser	Asn	Phe	Gln	Ala	Phe	Ala	Asp	Asp	Gln	Pro	Glu
		675				680						685			
Leu	Tyr	Met	Asn	Arg	Val	Leu	Ala	Lys	Asn	Thr	Asp	Leu	Leu	Lys	Ala
	690				695						700				
Trp	Gly	Val	Thr	Ser	Val	Gly	Leu	Pro	Pro	Gln	Tyr	Val	Ser	Ser	Lys
	705				710					715					720
Asp	Gly	Thr	Phe	Leu	Asp	Ser	Thr	Ile	Asp	Asn	Gly	Tyr	Ala	Phe	Asp
			725						730					735	
Asp	Arg	Tyr	Asp	Met	Ala	Leu	Ser	Gln	Asn	Asn	Lys	Tyr	Gly	Ser	Leu
		740						745					750		
Glu	Asp	Leu	Leu	Asn	Val	Leu	Arg	Ala	Leu	His	Lys	Asp	Gly	Ile	Gln
		755					760					765			
Ala	Ile	Ala	Asp	Trp	Val	Pro	Asp	Gln	Ile	Tyr	Asn	Leu	Pro	Gly	Lys
	770					775					780				
Glu	Val	Val	Asn	Ala	Thr	Arg	Val	Asn	Gly	Tyr	Gly	Tyr	His	Gln	Gln
	785				790					795				800	
Gly	Tyr	Gln	Ile	Val	Asp	Gln	Ala	Tyr	Val	Ala	Asn	Thr	Arg	Thr	Asp
			805						810					815	
Gly	Thr	Asp	Tyr	Gln	Gly	Arg	Tyr	Gly	Gly	Ala	Phe	Leu	Asp	Glu	Leu
			820					825					830		

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Lys Ala Lys Tyr Pro Ser Ile Phe Asn Arg Val Gln Ile Ser Asn Gly  
           835                                  840                                  845

Lys Gln Leu Pro Thr Asn Glu Lys Ile Thr Lys Trp Ser Ala Lys Tyr  
       850                                  855                                  860

Phe Asn Gly Thr Asn Ile Leu Gly Arg Gly Ile Asn Tyr Val Leu Arg  
   865                                  870                                  875                                  880

Asp Asp Lys Thr Asn Gln Tyr Phe Asn Thr Ser Ala Asn Gly Gln Leu  
                                   885                                  890                                  895

Leu Pro Thr Pro Leu Arg Asp Thr Gly Ala Ile Thr Ser Thr Gln Val  
                                   900                                  905                                  910

Phe Gln Arg Arg Gly Gln Asp Val Tyr Phe Leu Arg Asp Asn Gln Val  
       915                                  920                                  925

Ile Lys Asn Glu Phe Val Gln Asp Gly Asn Gly Asn Trp Tyr Tyr Phe  
       930                                  935                                  940

Gly Ala Asp Gly Lys Met Thr Lys Gly Ala Gln Asn Ile Asn Ser Lys  
   945                                  950                                  955                                  960

Asp Tyr Tyr Phe Phe Asp Asn Gly Val Gln Leu Arg Asn Ala Leu Arg  
                                   965                                  970                                  975

Arg Ala Ser Asn Gly Tyr Thr Tyr Tyr Tyr Gly Leu Asp Gly Ala Met  
                                   980                                  985                                  990

Ile Lys Asn Ala Phe Val Asp Phe Asp Asp Lys His Gln Gln Val Arg  
       995                                  1000                                  1005

Ala Phe Thr Thr Gln Gly Thr Met Val Val Gly Asn Leu His Trp  
   1010                                  1015                                  1020

Ser Gly His His Phe Tyr Phe Asp Arg Glu Thr Gly Ile Gln Ala  
   1025                                  1030                                  1035

Lys Asp Arg Ile Val Arg Thr Asp Asp Gly Lys Leu His Tyr Tyr  
   1040                                  1045                                  1050

Val Ala Gln Thr Gly Asp Met Gly Arg Asn Val Phe Ala Thr Asp  
   1055                                  1060                                  1065

Ser Arg Thr Gly Lys Arg Tyr Tyr Phe Asp Ala Asp Gly Asn Thr  
   1070                                  1075                                  1080

Val Thr Gly Ser Arg Val Ile Asp Gly Lys Thr Tyr Tyr Phe Asn  
   1085                                  1090                                  1095

Gln Asp Gly Ser Val Gly Thr Ala Tyr Ser Asn Arg Ala Asp Ser  
   1100                                  1105                                  1110

Ile Ile Phe Glu Asn Gly Lys Ala Arg Tyr Ile Thr Pro Ala Gly  
   1115                                  1120                                  1125

Glu Ile Gly Arg Ser Ile Phe Val Tyr Asn Pro Ala Thr Lys Ala  
   1130                                  1135                                  1140

Trp Asn Tyr Phe Asp Lys Glu Gly Asn Arg Val Thr Gly Arg Gln  
   1145                                  1150                                  1155

Tyr Ile Asp Gly Asn Leu Tyr Tyr Phe Lys Glu Asp Gly Ser Gln  
   1160                                  1165                                  1170

Val Lys Gly Ala Ile Val Glu Glu Asn Gly Ile Lys Tyr Tyr Tyr  
   1175                                  1180                                  1185

Glu Pro Gly Ser Gly Ile Leu Ala Ser Gly Arg Tyr Leu Gln Val  
   1190                                  1195                                  1200

Gly Asp Asp Gln Trp Ile Tyr Phe Lys His Asp Gly Ser Leu Ala  
   1205                                  1210                                  1215

Ile Gly Gln Val Arg Ala Asp Gly Gly Tyr Leu Lys Tyr Phe Asp  
   1220                                  1225                                  1230

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Lys Asn Gly Ile Gln Val Lys Gly Gln Thr Ile Val Glu Asp Gly  
1235 1240 1245

His Thr Tyr Tyr Tyr Asp Ala Asp Ser Gly Ala Leu Val Thr Ser  
1250 1255 1260

Ser Phe Ala Glu Ile Ala Pro Asn Gln Trp Ala Tyr Phe Asn Thr  
1265 1270 1275

Glu Gly Gln Ala Leu Lys Gly Lys Trp Thr Ile Asn Gly Lys Glu  
1280 1285 1290

Tyr Tyr Phe Asp Gln Asn Gly Ile Gln Tyr Lys Gly Lys Ala Val  
1295 1300 1305

Lys Val Gly Ser Arg Tyr Lys Tyr Tyr Asp Glu Asn Asp Gly Gln  
1310 1315 1320

Pro Val Thr Asn Arg Phe Ala Gln Ile Glu Pro Asn Val Trp Ala  
1325 1330 1335

Tyr Phe Gly Ala Asp Gly Tyr Ala Val Thr Gly Glu Gln Val Ile  
1340 1345 1350

Asn Gly Gln His Leu Tyr Phe Asp Gln Ser Gly Arg Gln Val Lys  
1355 1360 1365

Gly Ala Tyr Val Thr Val Asn Gly Gln Arg Arg Tyr Tyr Asp Ala  
1370 1375 1380

Asn Thr Gly Glu Tyr Ile Pro Gly Arg  
1385 1390

&lt;210&gt; SEQ ID NO 43

&lt;211&gt; LENGTH: 1455

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Streptococcus mutans

&lt;400&gt; SEQUENCE: 43

Met Glu Lys Lys Val Arg Phe Lys Leu Arg Lys Val Lys Lys Arg Trp  
1 5 10 15

Val Thr Val Ser Val Ala Ser Ala Val Val Thr Leu Thr Ser Leu Ser  
20 25 30

Gly Ser Leu Val Lys Ala Asp Ser Thr Asp Asp Arg Gln Gln Ala Val  
35 40 45

Thr Glu Ser Gln Ala Ser Leu Val Thr Thr Ser Glu Ala Ala Lys Glu  
50 55 60

Thr Leu Thr Ala Thr Asp Thr Ser Thr Ala Thr Ser Ala Thr Ser Gln  
65 70 75 80

Leu Thr Ala Thr Val Thr Asp Asn Val Ser Thr Thr Asn Gln Ser Thr  
85 90 95

Asn Thr Thr Ala Asn Thr Ala Asn Phe Asp Val Lys Pro Thr Thr Thr  
100 105 110

Ser Glu Gln Ser Lys Thr Asp Asn Ser Asp Lys Ile Ile Ala Thr Ser  
115 120 125

Lys Ala Val Asn Arg Leu Thr Ala Thr Gly Lys Phe Val Pro Ala Asn  
130 135 140

Asn Asn Thr Ala His Pro Lys Thr Val Thr Asp Lys Ile Val Pro Ile  
145 150 155 160

Lys Pro Lys Ile Gly Lys Leu Lys Gln Pro Ser Ser Leu Ser Gln Asp  
165 170 175

Asp Ile Ala Ala Leu Gly Asn Val Lys Asn Ile Arg Lys Val Asn Gly  
180 185 190

Lys Tyr Tyr Tyr Tyr Lys Glu Asp Gly Thr Leu Gln Lys Asn Tyr Ala  
195 200 205

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Leu Asn Ile Asn Gly Lys Thr Phe Phe Phe Asp Glu Thr Gly Ala Leu  
 210 215 220  
 Ser Asn Asn Thr Leu Pro Ser Lys Lys Gly Asn Ile Thr Asn Asn Asp  
 225 230 235 240  
 Asn Thr Asn Ser Phe Ala Gln Tyr Asn Gln Val Tyr Ser Thr Asp Ala  
 245 250 255  
 Ala Asn Phe Glu His Val Asp His Tyr Leu Thr Ala Glu Ser Trp Tyr  
 260 265 270  
 Arg Pro Lys Tyr Ile Leu Lys Asp Gly Lys Thr Trp Thr Gln Ser Thr  
 275 280 285  
 Glu Lys Asp Phe Arg Pro Leu Leu Met Thr Trp Trp Pro Asp Gln Glu  
 290 295 300  
 Thr Gln Arg Gln Tyr Val Asn Tyr Met Asn Ala Gln Leu Gly Ile His  
 305 310 315 320  
 Gln Thr Tyr Asn Thr Ala Thr Ser Pro Leu Gln Leu Asn Leu Ala Ala  
 325 330 335  
 Gln Thr Ile Gln Thr Lys Ile Glu Glu Lys Ile Thr Ala Glu Lys Asn  
 340 345 350  
 Thr Asn Trp Leu Arg Gln Thr Ile Ser Ala Phe Val Lys Thr Gln Ser  
 355 360 365  
 Ala Trp Asn Ser Asp Ser Glu Lys Pro Phe Asp Asp His Leu Gln Lys  
 370 375 380  
 Gly Ala Leu Leu Tyr Ser Asn Asn Ser Lys Leu Thr Ser Gln Ala Asn  
 385 390 395 400  
 Ser Asn Tyr Arg Ile Leu Asn Arg Thr Pro Thr Asn Gln Thr Gly Lys  
 405 410 415  
 Lys Asp Pro Arg Tyr Thr Ala Asp Arg Thr Ile Gly Gly Tyr Glu Phe  
 420 425 430  
 Leu Leu Ala Asn Asp Val Asp Asn Ser Asn Pro Val Val Gln Ala Glu  
 435 440 445  
 Gln Leu Asn Trp Leu His Phe Leu Met Asn Phe Gly Asn Ile Tyr Ala  
 450 455 460  
 Asn Asp Pro Asp Ala Asn Phe Asp Ser Ile Arg Val Asp Ala Val Asp  
 465 470 475 480  
 Asn Val Asp Ala Asp Leu Leu Gln Ile Ala Gly Asp Tyr Leu Lys Ala  
 485 490 495  
 Ala Lys Gly Ile His Lys Asn Asp Lys Ala Ala Asn Asp His Leu Ser  
 500 505 510  
 Ile Leu Glu Ala Trp Ser Tyr Asn Asp Thr Pro Tyr Leu His Asp Asp  
 515 520 525  
 Gly Asp Asn Met Ile Asn Met Asp Asn Arg Leu Arg Leu Ser Leu Leu  
 530 535 540  
 Tyr Ser Leu Ala Lys Pro Leu Asn Gln Arg Ser Gly Met Asn Pro Leu  
 545 550 555 560  
 Ile Thr Asn Ser Leu Val Asn Arg Thr Asp Asp Asn Ala Glu Thr Ala  
 565 570 575  
 Ala Val Pro Ser Tyr Ser Phe Ile Arg Ala His Asp Ser Glu Val Gln  
 580 585 590  
 Asp Leu Ile Arg Asn Ile Ile Arg Ala Glu Ile Asn Pro Asn Val Val  
 595 600 605  
 Gly Tyr Ser Phe Thr Met Glu Glu Ile Lys Lys Ala Phe Glu Ile Tyr  
 610 615 620

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Asn Lys Asp Leu Leu Ala Thr Glu Lys Lys Tyr Thr His Tyr Asn Thr  
 625 630 635 640  
 Ala Leu Ser Tyr Ala Leu Leu Leu Thr Asn Lys Ser Ser Val Pro Arg  
 645 650 655  
 Val Tyr Tyr Gly Asp Met Phe Thr Asp Asp Gly Gln Tyr Met Ala His  
 660 665 670  
 Lys Thr Ile Asn Tyr Glu Ala Ile Glu Thr Leu Leu Lys Ala Arg Ile  
 675 680 685  
 Lys Tyr Val Ser Gly Gly Gln Ala Met Arg Asn Gln Gln Val Gly Asn  
 690 695 700  
 Ser Glu Ile Ile Thr Ser Val Arg Tyr Gly Lys Gly Ala Leu Lys Ala  
 705 710 715 720  
 Thr Asp Thr Gly Asp Arg Thr Thr Arg Thr Ser Gly Val Ala Val Ile  
 725 730 735  
 Glu Gly Asn Asn Pro Ser Leu Arg Leu Lys Ala Ser Asp Arg Val Val  
 740 745 750  
 Val Asn Met Gly Ala Ala His Lys Asn Gln Ala Tyr Arg Pro Leu Leu  
 755 760 765  
 Leu Thr Thr Asp Asn Gly Ile Lys Ala Tyr His Ser Asp Gln Glu Ala  
 770 775 780  
 Ala Gly Leu Val Arg Tyr Thr Asn Asp Arg Gly Glu Leu Ile Phe Thr  
 785 790 795 800  
 Ala Ala Asp Ile Lys Gly Tyr Ala Asn Pro Gln Val Ser Gly Tyr Leu  
 805 810 815  
 Gly Val Trp Val Pro Val Gly Ala Ala Ala Asp Gln Asp Val Arg Val  
 820 825 830  
 Ala Ala Ser Thr Ala Pro Ser Thr Asp Gly Lys Ser Val His Gln Asn  
 835 840 845  
 Ala Ala Leu Asp Ser Arg Val Met Phe Glu Gly Phe Ser Asn Phe Gln  
 850 855 860  
 Ala Phe Ala Thr Lys Lys Glu Glu Tyr Thr Asn Val Val Ile Ala Lys  
 865 870 875 880  
 Asn Val Asp Lys Phe Ala Glu Trp Gly Val Thr Asp Phe Glu Met Ala  
 885 890 895  
 Pro Gln Tyr Val Ser Ser Thr Asp Gly Ser Phe Leu Asp Ser Val Ile  
 900 905 910  
 Gln Asn Gly Tyr Ala Phe Thr Asp Arg Tyr Asp Leu Gly Ile Ser Lys  
 915 920 925  
 Pro Asn Lys Tyr Gly Thr Ala Asp Asp Leu Val Lys Ala Ile Lys Ala  
 930 935 940  
 Leu His Ser Lys Gly Ile Lys Val Met Ala Asp Trp Val Pro Asp Gln  
 945 950 955 960  
 Met Tyr Ala Phe Pro Glu Lys Glu Val Val Thr Ala Thr Arg Val Asp  
 965 970 975  
 Lys Tyr Gly Thr Pro Val Ala Gly Ser Gln Ile Lys Asn Thr Leu Tyr  
 980 985 990  
 Val Val Asp Gly Lys Ser Ser Gly Lys Asp Gln Gln Ala Lys Tyr Gly  
 995 1000 1005  
 Gly Ala Phe Leu Glu Glu Leu Gln Ala Lys Tyr Pro Glu Leu Phe  
 1010 1015 1020  
 Ala Arg Lys Gln Ile Ser Thr Gly Val Pro Met Asp Pro Ser Val  
 1025 1030 1035  
 Lys Ile Lys Gln Trp Ser Ala Lys Tyr Phe Asn Gly Thr Asn Ile

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1040	1045	1050
Leu Gly Arg Gly Ala Gly Tyr 1055	Tyr Val Leu Lys Asp 1060	Gln Ala Thr Asn 1065
Thr Tyr Phe Ser Leu Val 1070	Ser Asp Asn Thr Phe 1075	Leu Pro Lys Ser 1080
Leu Val Asn Pro Asn His 1085	Gly Thr Ser Ser Ser 1090	Val Thr Gly Leu 1095
Val Phe Asp Gly Lys Gly 1100	Tyr Val Tyr Tyr Ser 1105	Thr Ser Gly Tyr 1110
Gln Ala Lys Asn Thr Phe 1115	Ile Ser Leu Gly Asn 1120	Asn Trp Tyr Tyr 1125
Phe Asp Asn Asn Gly Tyr 1130	Met Val Thr Gly Ala 1135	Gln Ser Ile Asn 1140
Gly Ala Asn Tyr Tyr Phe 1145	Leu Ser Asn Gly Ile 1150	Gln Leu Arg Asn 1155
Ala Ile Tyr Asp Asn Gly 1160	Asn Lys Val Leu Ser 1165	Tyr Tyr Gly Asn 1170
Asp Gly Arg Arg Tyr Glu 1175	Asn Gly Tyr Tyr Leu 1180	Phe Gly Gln Gln 1185
Trp Arg Tyr Phe Gln Asn 1190	Gly Ile Met Ala Val 1195	Gly Leu Thr Arg 1200
Val His Gly Ala Val Gln 1205	Tyr Phe Asp Ala Ser 1210	Gly Phe Gln Ala 1215
Lys Gly Gln Phe Ile Thr 1220	Thr Ala Asp Gly Lys 1225	Leu Arg Tyr Phe 1230
Asp Arg Asp Ser Gly Asn 1235	Gln Ile Ser Asn Arg 1240	Phe Val Arg Asn 1245
Ser Lys Gly Glu Trp Phe 1250	Leu Phe Asp His Asn 1255	Gly Val Ala Val 1260
Thr Gly Thr Val Thr Phe 1265	Asn Gly Gln Arg Leu 1270	Tyr Phe Lys Pro 1275
Asn Gly Val Gln Ala Lys 1280	Gly Glu Phe Ile Arg 1285	Asp Ala Asp Gly 1290
His Leu Arg Tyr Tyr Asp 1295	Pro Asn Ser Gly Asn 1300	Glu Val Arg Asn 1305
Arg Phe Val Arg Asn Ser 1310	Lys Gly Glu Trp Phe 1315	Leu Phe Asp His 1320
Asn Gly Ile Ala Val Thr 1325	Gly Ala Arg Val Val 1330	Asn Gly Gln Arg 1335
Leu Tyr Phe Lys Ser Asn 1340	Gly Val Gln Ala Lys 1345	Gly Glu Leu Ile 1350
Thr Glu Arg Lys Gly Arg 1355	Ile Lys Tyr Tyr Asp 1360	Pro Asn Ser Gly 1365
Asn Glu Val Arg Asn Arg 1370	Tyr Val Arg Thr Ser 1375	Ser Gly Asn Trp 1380
Tyr Tyr Phe Gly Asn Asp 1385	Gly Tyr Ala Leu Ile 1390	Gly Trp His Val 1395
Val Glu Gly Arg Arg Val 1400	Tyr Phe Asp Glu Asn 1405	Gly Val Tyr Arg 1410
Tyr Ala Ser His Asp Gln 1415	Arg Asn His Trp Asn 1420	Tyr Asp Tyr Arg 1425
Arg Asp Phe Gly Arg Gly 1430	Ser Ser Ala Ile 1435	Arg Phe Arg His 1440

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Ser Arg Asn Gly Phe Phe Asp Asn Phe Phe Arg Phe  
 1445 1450 1455

<210> SEQ ID NO 44

<211> LENGTH: 2715

<212> TYPE: DNA

<213> ORGANISM: Streptococcus mutans

<400> SEQUENCE: 44

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gtgaacggca aataactacta ctacaaagaa gatggaacat tacagaaaa ctacgcactg    60
aatatcaacg gcaaaacatt tttctttgat gaaacgggag cgttatccaa taacacattg    120
ccgagcaaaa aagccaacat cacgaacaac gataacacaa actcctttgc tcaatataac    180
caggtgtact caacggatgc agcgaatttt gaacatgtcg accattatct gacagccgaa    240
tcctgggatc gccctaaata catccttaa gatggaaaa catggacgca gtctacagaa    300
aaagacttta gaccgctgct tatgacgtgg tggcctgac aagaacaca acgccagtat    360
gtcaattaca tgaacgcca actgggcac catcagacat ataacacagc aacgagcccg    420
ctgcagctta atttagctgc ccaaacgatc cagacaaaa tcgaagaaa aatcacggct    480
gagaaaaata caaactggtt gagacaaacg atcagcgcat ttgttaaac acagtctgcg    540
tggaatagcg attctgaaaa accgtttgat gaccatttgc aaaaaggagc attggtgtat    600
tccaacaact caaaactgac gtcccaggca aattcaaac accgtatctt gaaccggaca    660
ccgacgaatc aaacaggcaa aaaagatcct agatatacgg cggaccgcac aattggcggg    720
tacgaatttc tgcttgctaa cgatgttgac aattctaacc cggttgtgca agccgaacag    780
ttgaactggc tgcattttct tatgaacttt ggaaacatct acgcgaacga tcctgacgct    840
aattttgatt caattagagt cgatgcgta gacaatggtg atgcagactt attgcaaatc    900
gcgggagatt atcttaaagc agcgaaggt attcataaaa acgataaagc tgccaatgac    960
catttaagca tcttggaagc atggctctat aatgatacac cgtacttaca tgatgacgga    1020
gataacatga tcaacatgga caaccgtttg cggctgagcc tgctttattc tttagccaaa    1080
ccggtgaacc agcgtagcgg catgaatcct ctgatcacia actctcttgt aaatcggacg    1140
gatgacaacg ctgaaacagc agcggttccg tcctattcat ttattagagc ccatgattct    1200
gaagtgcaag acctatcag aatattatc cgcgcagaaa ttaatcctaa cgtcgtaggc    1260
tactcattta cgatggaaga aatcaaaaa gcgtttgaaa tctacaacia agatttattg    1320
gctacagaga aaaaatatac gcattacaac acagcgttaa gctatgctct gcttttaacg    1380
aataaatcaa gcgtgccgag agtctattac ggcgatatgt ttacagatga cggacagtat    1440
atggctcata aaacgatcaa ctacgaagct atcgaaacat tgctgaaagc cagaattaa    1500
tatgtctctg gtggccaagc catgcgcaac caacaagtg gaaattccga aattatcacg    1560
tcagtcggtt atggcaaaag agcgtttaa gctacagata cgggcgacag aacaacgcgc    1620
acatcaggag tggcagtcac cgaaggcaat aaccgcgcc ttagattaa agcgtcagat    1680
cgcgttgtgg tcaacatggg agctgcccat aaaaatcagg cttatcggcc tcttttattg    1740
acaacggata atggcattaa agcctatcat tcagaccaag aagcagcggg tctggtcctg    1800
tacacgaacg atcggggcga acttatcttt acagctgccg acattaaagg atatgcaaat    1860
cctcaggttt caggctactt aggagtatgg gttcctgtgg gcgcagcggc tgatcaagac    1920
gtcagagtag ccgcatccac ggcgcgctca acagacggaa aaagcgtaca tcagaacgcg    1980
gctctggata gcccggttat gtttgaagc ttttctaact ttcaagcctt tgcaacgaaa    2040

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aaagaagaat acacaaaagt agttatcgca aaaaatgtag ataaatttgc ggaatgggga 2100
gttacggact ttgaaatggc gccgcagtat gtatcttcca cagatggcag ctttctggac 2160
tctgttatcc aaaacggata tgcatttacg gatagatacg accttggeat ctcaaacct 2220
aacaataacg gaacacggga tgacctggtt aaagccatca aagcaactca tagcaaaaggc 2280
attaaagtaa tggcagattg ggttccggac cagatgatg cgtttcctga aaaagaagtg 2340
gtcacagcta cgcgcgtaga taaatatggt acgcccgttg ctggcagcca aatcaaaaac 2400
acactgtacg tagttgatgg caaatcaagc ggaaaagacc aacaggccaa atatggaggt 2460
gcatttctgg aagaacttca agctaaatac cctgaacttt ttgcccgtaa acagatctct 2520
acaggagtgc cgatggaccc gtcctgcaaa atcaaacagt ggtcagcaaa atactttaac 2580
ggcacaaaaca tcttaggtcg gggcgcagga tatgtgttga aagaccaagc gacaaaacagc 2640
tactttagcc tgggtgtctga taatacattt ctgccgaaat cacttgtcaa tcctaaccat 2700
ggaacgtctt cctaa 2715

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&lt;210&gt; SEQ ID NO 45

&lt;211&gt; LENGTH: 904

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Streptococcus mutans

&lt;400&gt; SEQUENCE: 45

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Val Asn Gly Lys Tyr Tyr Tyr Tyr Lys Glu Asp Gly Thr Leu Gln Lys
1          5          10          15
Asn Tyr Ala Leu Asn Ile Asn Gly Lys Thr Phe Phe Phe Asp Glu Thr
20          25          30
Gly Ala Leu Ser Asn Asn Thr Leu Pro Ser Lys Lys Gly Asn Ile Thr
35          40          45
Asn Asn Asp Asn Thr Asn Ser Phe Ala Gln Tyr Asn Gln Val Tyr Ser
50          55          60
Thr Asp Ala Ala Asn Phe Glu His Val Asp His Tyr Leu Thr Ala Glu
65          70          75          80
Ser Trp Tyr Arg Pro Lys Tyr Ile Leu Lys Asp Gly Lys Thr Trp Thr
85          90          95
Gln Ser Thr Glu Lys Asp Phe Arg Pro Leu Leu Met Thr Trp Trp Pro
100         105         110
Asp Gln Glu Thr Gln Arg Gln Tyr Val Asn Tyr Met Asn Ala Gln Leu
115         120         125
Gly Ile His Gln Thr Tyr Asn Thr Ala Thr Ser Pro Leu Gln Leu Asn
130         135         140
Leu Ala Ala Gln Thr Ile Gln Thr Lys Ile Glu Glu Lys Ile Thr Ala
145         150         155         160
Glu Lys Asn Thr Asn Trp Leu Arg Gln Thr Ile Ser Ala Phe Val Lys
165         170         175
Thr Gln Ser Ala Trp Asn Ser Asp Ser Glu Lys Pro Phe Asp Asp His
180         185         190
Leu Gln Lys Gly Ala Leu Leu Tyr Ser Asn Asn Ser Lys Leu Thr Ser
195         200         205
Gln Ala Asn Ser Asn Tyr Arg Ile Leu Asn Arg Thr Pro Thr Asn Gln
210         215         220
Thr Gly Lys Lys Asp Pro Arg Tyr Thr Ala Asp Arg Thr Ile Gly Gly
225         230         235         240
Tyr Glu Phe Leu Leu Ala Asn Asp Val Asp Asn Ser Asn Pro Val Val

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245					250					255					
Gln	Ala	Glu	Gln	Leu	Asn	Trp	Leu	His	Phe	Leu	Met	Asn	Phe	Gly	Asn
			260					265					270		
Ile	Tyr	Ala	Asn	Asp	Pro	Asp	Ala	Asn	Phe	Asp	Ser	Ile	Arg	Val	Asp
		275					280					285			
Ala	Val	Asp	Asn	Val	Asp	Ala	Asp	Leu	Leu	Gln	Ile	Ala	Gly	Asp	Tyr
	290					295					300				
Leu	Lys	Ala	Ala	Lys	Gly	Ile	His	Lys	Asn	Asp	Lys	Ala	Ala	Asn	Asp
305					310					315					320
His	Leu	Ser	Ile	Leu	Glu	Ala	Trp	Ser	Tyr	Asn	Asp	Thr	Pro	Tyr	Leu
				325					330					335	
His	Asp	Asp	Gly	Asp	Asn	Met	Ile	Asn	Met	Asp	Asn	Arg	Leu	Arg	Leu
			340					345					350		
Ser	Leu	Leu	Tyr	Ser	Leu	Ala	Lys	Pro	Leu	Asn	Gln	Arg	Ser	Gly	Met
		355					360					365			
Asn	Pro	Leu	Ile	Thr	Asn	Ser	Leu	Val	Asn	Arg	Thr	Asp	Asp	Asn	Ala
	370					375					380				
Glu	Thr	Ala	Ala	Val	Pro	Ser	Tyr	Ser	Phe	Ile	Arg	Ala	His	Asp	Ser
385						390					395				400
Glu	Val	Gln	Asp	Leu	Ile	Arg	Asn	Ile	Ile	Arg	Ala	Glu	Ile	Asn	Pro
				405					410					415	
Asn	Val	Val	Gly	Tyr	Ser	Phe	Thr	Met	Glu	Glu	Ile	Lys	Lys	Ala	Phe
			420					425						430	
Glu	Ile	Tyr	Asn	Lys	Asp	Leu	Leu	Ala	Thr	Glu	Lys	Lys	Tyr	Thr	His
		435				440						445			
Tyr	Asn	Thr	Ala	Leu	Ser	Tyr	Ala	Leu	Leu	Leu	Thr	Asn	Lys	Ser	Ser
	450					455					460				
Val	Pro	Arg	Val	Tyr	Tyr	Gly	Asp	Met	Phe	Thr	Asp	Asp	Gly	Gln	Tyr
465						470					475				480
Met	Ala	His	Lys	Thr	Ile	Asn	Tyr	Glu	Ala	Ile	Glu	Thr	Leu	Leu	Lys
				485				490						495	
Ala	Arg	Ile	Lys	Tyr	Val	Ser	Gly	Gly	Gln	Ala	Met	Arg	Asn	Gln	Gln
			500					505					510		
Val	Gly	Asn	Ser	Glu	Ile	Ile	Thr	Ser	Val	Arg	Tyr	Gly	Lys	Gly	Ala
		515					520					525			
Leu	Lys	Ala	Thr	Asp	Thr	Gly	Asp	Arg	Thr	Thr	Arg	Thr	Ser	Gly	Val
	530					535					540				
Ala	Val	Ile	Glu	Gly	Asn	Asn	Pro	Ser	Leu	Arg	Leu	Lys	Ala	Ser	Asp
545						550					555				560
Arg	Val	Val	Val	Asn	Met	Gly	Ala	Ala	His	Lys	Asn	Gln	Ala	Tyr	Arg
				565					570					575	
Pro	Leu	Leu	Leu	Thr	Thr	Asp	Asn	Gly	Ile	Lys	Ala	Tyr	His	Ser	Asp
		580						585					590		
Gln	Glu	Ala	Ala	Gly	Leu	Val	Arg	Tyr	Thr	Asn	Asp	Arg	Gly	Glu	Leu
		595					600					605			
Ile	Phe	Thr	Ala	Ala	Asp	Ile	Lys	Gly	Tyr	Ala	Asn	Pro	Gln	Val	Ser
	610					615					620				
Gly	Tyr	Leu	Gly	Val	Trp	Val	Pro	Val	Gly	Ala	Ala	Ala	Asp	Gln	Asp
625						630					635				640
Val	Arg	Val	Ala	Ala	Ser	Thr	Ala	Pro	Ser	Thr	Asp	Gly	Lys	Ser	Val
				645					650					655	
His	Gln	Asn	Ala	Ala	Leu	Asp	Ser	Arg	Val	Met	Phe	Glu	Gly	Phe	Ser
			660					665					670		

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Asn Phe Gln Ala Phe Ala Thr Lys Lys Glu Glu Tyr Thr Asn Val Val  
           675                                  680                                  685  
 Ile Ala Lys Asn Val Asp Lys Phe Ala Glu Trp Gly Val Thr Asp Phe  
           690                                  695                                  700  
 Glu Met Ala Pro Gln Tyr Val Ser Ser Thr Asp Gly Ser Phe Leu Asp  
           705                                  710                                  715                                  720  
 Ser Val Ile Gln Asn Gly Tyr Ala Phe Thr Asp Arg Tyr Asp Leu Gly  
                                   725                                  730                                  735  
 Ile Ser Lys Pro Asn Lys Tyr Gly Thr Ala Asp Asp Leu Val Lys Ala  
                                   740                                  745                                  750  
 Ile Lys Ala Leu His Ser Lys Gly Ile Lys Val Met Ala Asp Trp Val  
           755                                  760                                  765  
 Pro Asp Gln Met Tyr Ala Phe Pro Glu Lys Glu Val Val Thr Ala Thr  
           770                                  775                                  780  
 Arg Val Asp Lys Tyr Gly Thr Pro Val Ala Gly Ser Gln Ile Lys Asn  
           785                                  790                                  795                                  800  
 Thr Leu Tyr Val Val Asp Gly Lys Ser Ser Gly Lys Asp Gln Gln Ala  
                                   805                                  810                                  815  
 Lys Tyr Gly Gly Ala Phe Leu Glu Glu Leu Gln Ala Lys Tyr Pro Glu  
                                   820                                  825                                  830  
 Leu Phe Ala Arg Lys Gln Ile Ser Thr Gly Val Pro Met Asp Pro Ser  
           835                                  840                                  845  
 Val Lys Ile Lys Gln Trp Ser Ala Lys Tyr Phe Asn Gly Thr Asn Ile  
           850                                  855                                  860  
 Leu Gly Arg Gly Ala Gly Tyr Val Leu Lys Asp Gln Ala Thr Asn Thr  
           865                                  870                                  875                                  880  
 Tyr Phe Ser Leu Val Ser Asp Asn Thr Phe Leu Pro Lys Ser Leu Val  
                                   885                                  890                                  895  
 Asn Pro Asn His Gly Thr Ser Ser  
                                   900

&lt;210&gt; SEQ ID NO 46

&lt;211&gt; LENGTH: 1308

&lt;212&gt; TYPE: DNA

<213> ORGANISM: *Penicillium marneffeii*

&lt;400&gt; SEQUENCE: 46

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atgaagcaaa ccacttcct ctcctctca gccatcgcg caaccagcag cttcagcgga    60
ctaacagccg ctcaaaaact cgctttgcg cacgtcgtcg tcggcaacac tgcagcacac    120
acccaatcca cctgggaaag cgacattact ctgcgccata actccggtct agatgccttt    180
gccttgaacg gtggattccc cgatggcaac atccccgcac aaatcgccaa cgcttttgcg    240
gcttggaag ccctttcaaa tggcttcaag ctattcattt cgtttgacta cctcggtggt    300
ggtcagccct ggctgcctc agaggttggt tctatgctga agcagtatgc cagttccgat    360
tgttatttgg cctatgatgg caagcccttt gtctcaactt ttgagggcac cggaaatatt    420
gcggtattgg gcacaggagg tccattcgg tcggcggtgg atgtttactt tgtgccggat    480
tggacgagtt tggggcctgc tgggattaag tcgtatctcg acaatatcga tggatttttc    540
agctggaaca tgtggcctgt aggtgcggcc gatatgaccg acgagcctga tttcgaatgg    600
ctcgatgcaa ttgggtccga caagacgtac atgatgggag tttcgccatg gttcttcac    660
agtgaacgag gaggcaccga ctgggtctgg cgtggtgatg acctctggga tgaccgatgg    720
attcaagtca cctgcgtcga cctcaattt gtccaggtcg tcacatggaa cgactggggg    780

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gaatcctcct acatcgggccc ctctgtgacc gctagcgaag tccccgccgg ctcattagcc 840
tacgtogaca acatgtcaca ccaaagcttc cttgacttct tgcctttcta catcgccacc 900
ttcaaaggcg acacattcaa catctcccgc gaccagatgc aatactggta cgcctogca 960
cccgccgcag caggcagcgc gtgcggcgta tacggcaatg atcccgatca aggccagact 1020
accgttgacg tcaactccat cgttcaggac aaggtgtttt tcagtgtttt gttgacggct 1080
gatgtactg taacgggtga gattgtagt aatgctgctg tttcatatga tgggtttgct 1140
ggtatgaacc actggagtca ggactttaat ggccagaccg gcgcggttac gtttagtggt 1200
gtcaggggtg gcgctacagt taagagtggg attggagccg agattacggc ttcgacttcg 1260
ttgtcgaatg ggtgcactaa ttacaaccct tgggttggtg gtttctaa 1308

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&lt;210&gt; SEQ ID NO 47

&lt;211&gt; LENGTH: 435

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Penicillium marneffeii*

&lt;400&gt; SEQUENCE: 47

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Met Lys Gln Thr Thr Ser Leu Leu Leu Ser Ala Ile Ala Ala Thr Ser
1          5          10          15
Ser Phe Ser Gly Leu Thr Ala Ala Gln Lys Leu Ala Phe Ala His Val
20        25        30
Val Val Gly Asn Thr Ala Ala His Thr Gln Ser Thr Trp Glu Ser Asp
35        40        45
Ile Thr Leu Ala His Asn Ser Gly Leu Asp Ala Phe Ala Leu Asn Gly
50        55        60
Gly Phe Pro Asp Gly Asn Ile Pro Ala Gln Ile Ala Asn Ala Phe Ala
65        70        75        80
Ala Cys Glu Ala Leu Ser Asn Gly Phe Lys Leu Phe Ile Ser Phe Asp
85        90        95
Tyr Leu Gly Gly Gly Gln Pro Trp Pro Ala Ser Glu Val Val Ser Met
100       105       110
Leu Lys Gln Tyr Ala Ser Ser Asp Cys Tyr Leu Ala Tyr Asp Gly Lys
115       120       125
Pro Phe Val Ser Thr Phe Glu Gly Thr Gly Asn Ile Ala Asp Trp Ala
130       135       140
His Gly Gly Pro Ile Arg Ser Ala Val Asp Val Tyr Phe Val Pro Asp
145       150       155       160
Trp Thr Ser Leu Gly Pro Ala Gly Ile Lys Ser Tyr Leu Asp Asn Ile
165       170       175
Asp Gly Phe Phe Ser Trp Asn Met Trp Pro Val Gly Ala Ala Asp Met
180       185       190
Thr Asp Glu Pro Asp Phe Glu Trp Leu Asp Ala Ile Gly Ser Asp Lys
195       200       205
Thr Tyr Met Met Gly Val Ser Pro Trp Phe Phe His Ser Ala Ser Gly
210       215       220
Gly Thr Asp Trp Val Trp Arg Gly Asp Asp Leu Trp Asp Asp Arg Trp
225       230       235       240
Ile Gln Val Thr Cys Val Asp Pro Gln Phe Val Gln Val Val Thr Trp
245       250       255
Asn Asp Trp Gly Glu Ser Ser Tyr Ile Gly Pro Phe Val Thr Ala Ser
260       265       270
Glu Val Pro Ala Gly Ser Leu Ala Tyr Val Asp Asn Met Ser His Gln
275       280       285

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Ser Phe Leu Asp Phe Leu Pro Phe Tyr Ile Ala Thr Phe Lys Gly Asp  
 290 295 300

Thr Phe Asn Ile Ser Arg Asp Gln Met Gln Tyr Trp Tyr Arg Leu Ala  
 305 310 315 320

Pro Ala Ala Ala Gly Ser Ala Cys Gly Val Tyr Gly Asn Asp Pro Asp  
 325 330 335

Gln Gly Gln Thr Thr Val Asp Val Asn Ser Ile Val Gln Asp Lys Val  
 340 345 350

Phe Phe Ser Ala Leu Leu Thr Ala Asp Ala Thr Val Thr Val Gln Ile  
 355 360 365

Gly Ser Asn Ala Ala Val Ser Tyr Asp Gly Val Ala Gly Met Asn His  
 370 375 380

Trp Ser Gln Asp Phe Asn Gly Gln Thr Gly Ala Val Thr Phe Ser Val  
 385 390 395 400

Val Arg Gly Gly Ala Thr Val Lys Ser Gly Ile Gly Ala Glu Ile Thr  
 405 410 415

Ala Ser Thr Ser Leu Ser Asn Gly Cys Thr Asn Tyr Asn Pro Trp Val  
 420 425 430

Gly Ser Phe  
 435

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What is claimed is:

1. A method that comprises:

- (a) providing a glucan synthesis reaction;
- (b) obtaining a fraction of the glucan synthesis reaction that comprises a saccharide byproduct of the glucan synthesis reaction, wherein the saccharide byproduct is a disaccharide or oligosaccharide and comprises at least one alpha-1,3 glucosyl-glucose linkage; and
- (c) contacting the saccharide byproduct in the fraction with an alpha-glucosidase enzyme, wherein the alpha-

- glucosidase enzyme hydrolyzes at least one alpha-1,3 glucosyl-glucose linkage of the saccharide byproduct.
- 2. The method of claim 1, wherein the alpha-glucosidase enzyme is immobilized.
- 3. The method of claim 1, wherein the degree of polymerization of the saccharide byproduct before hydrolysis is 3 to 7.
- 4. The method of claim 1, wherein the alpha-glucosidase enzyme is a transglucosidase.

\* \* \* \* \*